RENAL MECHANISMS CONCERNED IN THE CONTROL OF BLOOD VOLUME

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, Renal Mechanisms Concerned in the Control of Blood Volume, submitted by Edward Laurence Atkins, B.Sc., M.D., in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

In order to study afferent pathways and effector mechanisms of a reflex controlling blood volume, 18 dogs were anaesthetized with pentobarbital and given 6% bovine albumin in Ringer-Locke solution or canine plasma infusions of at least 15% of the total estimated blood volume. This resulted in a water and salt diuresis in every case except one. Renal function was measured by determining the exogenous creatinine clearance (GFR) and p-aminohippurate clearance (RPF). Urinary excretion of sodium was estimated by flame photometry. The relationship between chenges in right atrial and arterial pressures and increased urine flow was not consistent. The changes in GFR were variable and there was no apparent change with the diuresis but there was a tendency for the RPF to increase during the diuresis.

The increase in sodium output appeared to precede the water diuresis by 10 to 20 minutes and it is suggested that two separate mechanisms, independently controlling water and salt excretion, were operating. This view was strengthened by the results of a small series of adrenalectomized dogs maintained on DCA and cortisone, in which the water response to infusion was not altered but the sodium response was decreased. All the

HISTORICAL REVIEW

Introduction

Starling in 1909 was probably the first to suggest that the kidney might be the organ through which blood volume is controlled. Peters (1935) suggested that the "fullness of the blood stream might provoke the diuretic response to the kidneys." He did not suggest that the kidney might excrete more water as a direct result of increased volume but rather that it maintained fluid volume by excreting or retaining osmotically active solutes.

Since Verney provided evidence that osmo-receptors controlled the level of the osmotic pressure of the extracellular fluid by regulating the secretion of anti-diuretic hormone acting on the renal tubules, there has been increasing speculation and search ~~of~~ for similar mechanisms controlling blood volume. Certain unexplained phenomena suggest that the kidneys are the effector organs for these mechanisms. As will be shown later, there are many reports of iso-osmotic infusions being followed by an increase in urine volume. The osmo-receptors could not have been affected and yet compensations, presumably for the increased intra-vascular volume, resulted in an increase in the renal loss of water. The evidence for such a mechanism will be discussed as well as the possible modes of action.

With the assumption that a means for controlling blood volume exists, a summary has been compiled of the present literature dealing with hypothesized mechanisms. The literature will be discussed under the following headings: suggested sites and anatomical evidence of an hypothesized volume receptor; afferent pathways of such a receptor; reports of variations of activity of appropriate receptors and accompanying effects on renal function; evidence for possible effector mechanisms and finally a brief review of other factors that may indirectly influence blood volume.

The Volume Receptor

Possible Sites and Anatomical Evidence

It is conceivable that changes in extracellular fluid volume could be registered as some parameter of the intravascular compartment and the most logical mechanism for this would be a receptor that is stretch-sensitive. Several sites, such as the thorax, heart and head have been postulated as containing these receptors. The evidence for the various locations of these receptors will be discussed.

As a result of their work with negative pressure breathing (NPB), which tends to distend the intra-thoracic viscera, Gauer, Henry, Sieker and Wendt (1951) suspected the heart and lungs as containing a possible receptor organ for controlling blood volume. NPB in dogs anaesthetized with morphine and chloralose resulted in an increased urine flow (130%)

while positive pressure breathing was followed by a decreased urine flow. The hypothetical receptor pathways were blocked at the lung hilus with 1% procaine and the response was abolished in two cases, reduced in two cases and unchanged in one case. While this would seem to implicate the thoracic viscera as the sensitive organ, the same group, Henry, Gauer, Sieker, Wendt and Reeves (1953) ruled out the pulmonary vascular tree by causing vascular hypertension and noting no change in urine flow. Small plastic beads were injected intravenously in six dogs on the assumption that this would block circulation and cause pulmonary hypertension. The failure of this procedure to cause an increase in urine flow ruled out the possibility of the volume receptors responsible for the diuresis being in the pulmonary tree but did not exclude their location elsewhere in the thorax.

The same workers, in 1952, suggested that the volume receptors were in the low-pressure side of the cardiovascular system. Variations in the central venous, right atrial, pulmonary arterial and left atrial pressures were measured during hemorrhage or infusion of blood. Pressure changes in the left atrium, proportional to blood volume alterations, were greatest and they concluded that this chamber was the most likely site for a volume receptor.

Henry, Gauer and Reeves (1954 and 1956) summarized the effects of various procedures on the heart and were able to

localize the site of the probable thoracic volume receptor. Neither obstruction of the pulmonary arterioles by plastic beads nor obstruction of the pulmonary veins by snares produced a diuresis in dogs. Distension of a balloon placed in the left atrium resulted in a diuresis 2-5 times control levels. This was accompanied by little change in arterial or right atrial pressures. The diuresis returned to normal in 30 minutes despite continued distention. They came to the conclusion that the stretch receptors were most likely to be in the left atrium and perhaps the right was involved as well. They believed that these stretch receptors were instrumental in linking changes in blood volume with homeostatic responses of the kidney.

Anatomical evidence of stretch receptors in the atria has been provided by Nonidez (1937, 1941) who found definite receptor areas in the intrapericardial portion of the venae cavae and of the pulmonary veins. They were not found in the ventricles. He felt that their construction made them appropriate to measure the "minor changes in pressure characteristic of the venous system." More recently, this was confirmed by Coleridge, Hemingway, Holmes and Linden (1957) who studied histologically the sites of origin of action potentials from atrial stretch receptors.

Thus indirect evidence of stretch receptors in the atria is provided by NPB as well as histological evidence of their location. It is suggested that these receptors may have a function in controlling blood volume.

Other sites for a volume receptor have been suggested in the past and these will be considered. Because an unmistakable diuresis occurred in the recumbent subject shortly after a hypotonic saline infusion but did not occur in the sitting subject, Strauss, Davis, Rosenbaum and Rossmeisel (1951, 1952) concluded that the volume receptor was in the cephalad portion of the body. It is interesting to note that sodium excretion rose in the former group but water was excreted in excess of solutes which would suggest that inhibition of anti-diuretic hormone was responsible.

Lewis, Buie, Sevier and Harrison (1950) found that sodium excretion was less in sitting than in recumbent subjects. Compression of the neck with a cuff at 15-35 mm. of Hg to reduce the blood flow from the head, partially overcame this difference in sodium excretion. Viar,et al. (1951) did a similar study with compression of the neck and noted a similar increase in sodium excretion but no change in urine flow. They hypothesized the existence of an intra-cranial mechanism for controlling the extracellular fluid volume.

In contrast, Fishman in 1953, was unable to find any alteration in urine flow, sodium excretion, or creatinine or PAH clearance on compression of the neck in animals. He concluded that there was no evidence for the presence of receptors controlling blood volume in the head or neck region. Bull (1952) also failed to find any increase in urine flow in patients with increased intra-cranial pressure.

There have been other more remote suggestions of the possible sites of a volume receptor such as in the legs, but much of the conflicting evidence can be reconciled if one considers that all the above manoeuvers alter thoracic blood content in one way or another.

Although there is some suggestion that there are volume receptors in other parts of the body, the evidence for their location in the atria is much greater. They have been demonstrated microscopically by Nonidez and Hemingway and have been shown to be sensitive to stretch. The evidence that they could respond to changes in blood volume is good if it is considered that mechanical stimulation and NPB resemble increases in blood volume.

Afferent Pathways of Atrial Receptors

From such a receptor organ as has been mentioned, afferent fibers must carry impulses to the brain in the sensory side of a reflex arc. Two nervous pathways are conceivable; afferent fibers of the vagus and the dorsal roots T1-4, carrying sympathetic afferents. There is considerable evidence that the former is exclusively the case.

Whitteridge (1948) demonstrated the existence of afferents in the vagus nerve which showed increased activity during periods of increased cardiac filling. These fibers were shown later by Paintal (1953), in the cat, to arise from receptors in the atria, and to respond in direct proportion to the atrial

volume. These receptors have also been shown to exist in the dog (Pearce and Henry, 1954) and to respond by increased activity to inflation of the indwelling left atrial balloon (Henry and Pearce, 1956). Additional methods of stimulation, viz., NPB, infusion of blood, 6% bovine albumin in saline and isotonic saline gave variable but consistent increases in activity. The diuretic response to balloon inflation was abolished in six experiments by cold-blocking the vagi at a temperature also shown to block conduction in the atrial fibers. The diuresis of NPB was also abolished or reduced by cold-blocking of the vagi in seven dogs (Henry, et al., 1953).

Pathways in the dorsal roots were sectioned by thoracic sympathectomy in dogs (Henry, et al., 1953) but NPB was still followed by a diuresis. Pearce, Henry and Chapman (1956) could not find any fibers in the dorsal roots T1-T4 that carried a cardiac rhythm. This was done on only one dog and it is possible but unlikely that small unmyelinated fibers which were not investigated, could have carried the impulses.

There is now adequate electrophysiological evidence that atrial receptors, responding to atrial distention, have afferent fibers in the vagus nerve. The possibility still exists that these receptors have alternate pathways, or that other receptors of volume changes exist in the chest but the weight of evidence is in favor of the above conclusion. From the above evidence it can be fairly safely stated that there are receptors

in the atria which are capable of signalling the brain when stimulated by any manoeuver leading to stretch of the atrial walls. It is possible that such a mechanism could influence urine flow when blood volume is altered but complete evidence is lacking.

Renal Responses to Increased Stimulation of the "Volume Receptor"

The volume receptors can be stimulated in a variety of ways in an effort to observe the effects of artificially increasing the blood volume. An increased excretion of water and electrolytes would be the expected result and it will be shown later that this is probably the case. Mechanical stimulation of the atria is the most positive way of stimulating the "volume receptor" and the infusion of volume-expanding fluids is the most physiological method. Both of these methods will be reviewed.

Mechanical Stimulation of the Atria

The use of negative pressure breathing, ligatures and inflation of balloons are techniques that have been used to stimulate the atrial receptors.

Negative pressure breathing has been shown to cause engorgement of the heart and pulmonary circulation (Gauer, et al., 1954) and this would include the atria. Gauer, Henry, Sieker and Wendt (1951, 1954) studied the effect of negative pressure breathing on dogs anaesthetized with morphine and chloralose. A negative pressure

of -10 cm. of water was applied to the dogs and this resulted in a 100-200% increase in urine flow with the peak in 30-40 minutes. In 27 out of 70 experiments the urine flow did not return to normal but stayed 30% above normal.

Sieker, Gauer and Henry (1952, 1954) studied the effect of NPB on man and its effect on renal function. As well as a diuresis they noticed that the concentration of sodium in the urine decreased 73% but the total sodium increased only 17%. This suggests that the increase in urine volume was primarily a water diuresis. The glomerular filtration rate (GFR) after a brief initial increase, returned to normal and they concluded that this did not significantly affect the diuresis. The possibility that the diuresis was due to alkalosis was ruled out by the absence of urinary pH changes or respiratory rate. Thus it was demonstrated that NPB increased water output, probably by increasing some thoracic vascular parameter.

Another more direct method of stimulating the atrial receptors is by the use of ligatures to impede the blood flow distal to the pulmonary veins and stretching of the atria by inflation of balloons (Henry, Gauer and Reeves, 1954 and 1956). Tensing ligatures around the pulmonary veins resulted in a diuresis only when they were around the intrapericardial portion of the vein. This left the possibility that the left atrium was stretched as well but when care was taken not to raise the pressure in the atrium, a diuresis did not result.

Distention of a balloon in the left atrium produced an increase in left atrial and pulmonary pressure and in 37 balloon distentions in 13 dogs, the urine flow increased in 14 trials. It is interesting to note that the urine flow returned to normal in 30 minutes despite continued distention. They were thus led to suggest that stretch receptors in the left atrium were responsible for the increased urine flow observed.

It should be noted that mechanical stimulation of the atria has little effect on the sodium output and as will be shown later, a naturesis is characteristic of more physiological methods of increasing blood volume. This indicates that the diuretic reflex mentioned above may be different from or only part of a mechanism for controlling blood volume.

Stimulation by Increasing the Blood Volume

Isotonic and isosmotic infusions increase the total blood volume and presumably stimulate atrial stretch receptors as well. A slow infusion can be likened to a physiological expansion of the blood volume and the effects of the blood volume expansion with isotonic saline, dextran, bovine albumin, plasma and blood will be considered in turn. Changes in urine and sodium output are indications of their effects and it is these changes in which we are primarily interested.

(i) Saline infusions

Infusions of isotonic saline or Ringer-Locke solution are known to expand the blood volume for limited periods of time. They diffuse into the tissues fairly rapidly and because they lack proteins, it may be argued that they are not physiological. Homer Smith (1938) reports that saline raises the glomerular filtration rate (GFR) in dogs but not in man. This species difference indicates that the mechanism for blood volume control might not be comparable. It is difficult to believe that the mechanisms in dog and man are greatly different but an increased GFR is one method that must be considered for controlling blood volume.

Wesson, et. al. (1950) used Locke's solution as an infusate to expand the extracellular fluid volume in trained unanaesthetized dogs and found a three-phase response with an increase in the GFR, renal plasma flow (RPF) and filtration fraction (FF) as well as urine flow. They concluded that changes in renal hemodynamics accounted for the diuresis and the observed increase in sodium excretion.

Strauss, Davis, Rosenbaum and Rossmeisl (1951) infused isotonic saline into normal subjects in large quantities (3 liters) and produced a water diuresis in recumbent subjects but not in those sitting. From this they concluded that the hypothetical volume receptor was in the cephalad portion of the body. Although the rate of sodium and chloride excretion rose, the concentration fell and because this is characteristic of a diuresis

due to water, they concluded that the supra-optico-hypophyseal system had been responsible. This suggests that the distribution as well as the volume are important in producing a diuretic response.

Normally hydrated sitting subjects show no water diuresis following the ingestion of two liters of normal saline (Birchard and Strauss, 1953). However if the subject is on the descending limb of a water diuresis, a similar amount of saline will produce a slight augmentation of urine flow. A previous intake of sodium will have a similar effect. It would appear from this that prehydration determines whether or not a diuresis occurs.

In contrast to the effect shown in man, infusion of saline in dogs is followed by a prompt saline diuresis and as Homer Smith states, an increase in the GFR. These variable effects on urine flow with saline make it a poor substance to study expansion of the blood volume. There is a suggestion too that the diuresis it produces differs from that caused by other substances.

(ii) Dextran

Since the development of blood substitutes such as the polysaccharides of large molecular weight (Dextran) it was hoped that they would present an easy method of expanding blood volume to study renal function changes. They would have the advantages of being free of unknown hormones and storage difficulties. Dextran has the disadvantage that some of the smaller molecules

pass through the glomerular filter and by increasing the osmotic pressure in the tubules, an osmotic diuresis could account for any changes in urine flow which might occur. This additional variable makes it unsuitable for volume expansion studies, however some important work has been done using this material and it bears reporting.

Wasserman and Mayerson (1952) showed that Dextran infusions are more efficient in bled dogs than in unbled dogs because the expansion of the plasma volume was greater in the former group. Infusion of large doses (40 ml./kg.) resulted in a diuresis in unbled dogs that was eight times control while the same amount in bled dogs produced only a slight diuresis. Interestingly enough, when the smaller molecules were removed from the infusate, there was no diuresis in non-bled dogs. This would seem to indicate that the diuresis is due to the renal-excretable portion of the Dextran. However, other workers (Fleming, Cargill and Bloom, 1952) reported that infusions of Dextran caused no change in the GFR which is evidence against renal-excretable molecules causing a diuresis.

Gowdey and Young (1953, 1954) infused Dextran into dogs until death occurred or until the infusion exceeded the blood volume. They found that the GFR and urine flow varied whereas the RPF increased. When much smaller amounts were infused the total peripheral resistance, GFR and renal blood flow decreased and there was no correlation between right auricular pressure and cardiac output. The observed hemodilution

was consistently greater than that expected from the infusion, because the dextran solution was presumably hypertonic .

Recently, Young, Pearce and Stevenson (1955) used dextran as well as plasma and blood to expand the blood volume in an effort to study changes in renal function that might take place to result in a diuresis. An infusion of 6% dextran consistently gave a diuresis in dogs anaesthetized with pentobarbital which was associated with an insignificant change in the creatinine clearance (GFR) and a slight increase in the right auricular pressure and renal blood flow. Their results with plasma and whole blood will be discussed under the appropriate headings.

Dextran administration appears to be a good method by which to expand the blood volume and observe changes in body function. However, it has the disadvantage that the smaller molecules pass through the glomerular filter and this would introduce further variables in a comparison with more physiological substances.

(iii) Albumin and Gum Acacia

Materials such as 6% albumin in Ringer-Locke solution and gum acacia have been used as plasma expanders because of their ease of preparation and their freedom from unknown hormones. The influence of gum acacia in 5% glucose on renal function was studied in dogs by Goudsmit, Power and Bollman (1934). No significant difference was found in the colloid osmotic pressure when gum acacia was given. The GFR did not change but the rate of chloride excretion increased 190%. Gum acacia has the disadvantage that

it is toxic to the liver if given over long periods of time and this is one reason for its discontinued use.

The reports using albumin solutions to expand plasma volume are more difficult to interpret because often the infusates were not isosmotic. Hyperoncotic solutions such as used by Petersdorf and Welt (1953) would expand the intravascular volume but some of this expansion would be at the expense of the extra- or intracellular fluid. This would probably cause other changes in homeostatic mechanisms. For example, when hyperoncotic albumin was infused into normal subjects, an anti-diuresis resulted. This was to be expected for the hyperoncotic infusate would raise the osmotic pressure of the blood and hence probably stimulate release of excess anti-diuretic hormone. When similar solutions were infused into diabetes insipidus subjects the anti-diuresis was less but still present to some degree. It would seem that the patients either had some functioning posterior pituitary remaining or some other mechanism was responsible. The authors suggested that there was an organ in the vascular tree sensitive to changes in oncotic pressure.

Pre-hydrated subjects that were given concentrated salt-poor albumin (Goodyer, Peterson and Relman, 1949) also had a reduced urine flow as well as decreased excretion rates of sodium and chloride and a decreased mannitol clearance. These authors concluded that the concentrated albumin increased the excretion of anti-diuretic hormone. Cargill (1948) observed the effect of rapid injection of large amounts of albumin on renal

function. Inulin and para-amino hippurate clearances increased with infusion of 25% salt-poor albumin, but the rapid rate of infusion may have affected the results. By using a similar technique, Orloff and Blake (1951) observed a diuresis in animals that had been previously hydrated. When hydropenic animals were used, the urine flowwas still increased but less consistently. The creatinine clearance remained unchanged. Since albumin may act by inhibiting the excretion of ADH they suggested that further experiments be carried out on hypophysectomized dogs.

The work of Welt and Orloff (1949 and 1951) now becomes especially interesting. They studied the effects of various concentrations of salt-poor and iso-oncotic albumin on the excretion of water and electrolytes of normal subjects. Hyper-oncotic albumin (10-25%), although it increased the plasma volume, did not increase water excretion but sodium excretion decreased without a change in the endogenous creatinine clearance. Iso-oncotic albumin caused a diuresis and only a questionable change in the excretion of sodium. That activation of the supra-optico-hypophyseal system might be responsible for the lack of a diuresis when concentrated solutions were infused was discarded by them on the grounds that an anti-diuresis did not always result, and that the osmoreceptors were probably located outside the vascular tree. The adrenal cortex as a factor in this mechanism was made unlikely by the absence of any change in the above results when a patient with controlled Addison's disease was infused. They suggested the possibility of an onco-receptor which when stimulated

by hyperoncotic solutions, resulted in an increased reabsorption of sodium, followed by water. They postulated that the uncomplicated expansion of the plasma volume initiates a diuresis of water by suppressing the activity of the posterior pituitary gland and that an increase in the colloid osmotic pressure of the plasma resulted in an increase in the tubular reabsorption of sodium through some unknown mechanism which might possibly be a volume-receptor or some parameter of an abnormal volume.

The effect of vagotomy on the diuresis produced by infusions of isosmotic bovine albumin in Ringer-Locke solution was studied by Pearce and Roberts (1954). Immediately after section of the vagus, there was a brief anti-diuresis accompanied by a rise in blood pressure. Carotid sinus denervation as well as vagotomy produced an anti-diuresis and diminution in the total chloride excretion. Although a diuresis still followed an infusion after vagotomy, they believed at first that its character was slightly changed to a two-phased type. Later, they concluded that vagotomy had no effect on the diuresis. The failure to abolish the diuretic response by vagotomy, or carotid sinus denervation would suggest that hypothetical volume receptors do not use these afferent pathways. This strongly suggests that the atria are not volume receptors. However, there are arguments which leave these results in doubt. Albumin, although it is very similar to plasma especially when it is dissolved in Ringer-Locke solution, is not exactly comparable to a physiological expansion of the blood volume. It may contain antigens and it could be argued that it may stimulate receptors other than volume receptors to produce a diuresis.

(iv) Plasma and blood

The most physiological way of expanding the intravascular volume is by the infusion of plasma or better still, whole blood. We could then be certain that tonicity of the blood had not changed and if blood were used, even the proportion of formed elements would not be altered. There are a few early reports of blood having been given to note the effect on renal function. In 1930, Marx noticed that when blood was infused from a fasting dog into another dog, the recipient did not have a diuresis, while blood from a water-loaded dog did cause a diuresis. A red cell concentrate, when infused into dogs to study the effects of hypervolemia on the circulation, was (Gregg and Wiggers, 1933) also noticed to be followed by an increased urine flow, without any change in heart rate, blood pressure, venous pressure or splenic volume but with an increase in cardiac output as well as the size of the blood reservoirs. Natravisesh and White (1950) followed hemorrhage in dogs by transfusion and found that the GFR and RPF did not change. Corcoran and Page (1943) did a similar experiment and found that a transfusion would restore the diodrast clearance to normal.

More recently, Wilson and Harrison (1950) studied renal effects of large rapid infusions of plasma in convalescent men. This resulted in a marked rise in the clearance of creatinine and PAH and a diuresis resulted but when the infusion was given at a slower rate, the clearances were more nearly normal.

Metcalf (1944), while studying the effect of transfused plasma and serum in dogs, found that the protein disappeared from the blood stream at a constant rate but did not appear in the urine. The rate of disappearance was constant and was independent of the amount given. It was also found that the diuresis was proportional to the increase in the plasma volume. The red blood cells decreased in volume which is difficult to explain if the infused material was isotonic as should be expected.

Henry and Gauer (1951) who were the first to postulate that changes in blood volume are detected by nervous receptors in the thoracic viscera, which induce appropriate changes in the peripheral vascular bed and in renal function, came to this conclusion after infusing dogs with plasma or causing hemorrhages small enough to leave the blood pressure undisturbed. An anti-diuresis followed hemorrhage together with a decrease in the size of the heart and a decrease in lung density. This was good evidence that physiological changes in the blood volume affected changes in urine flow.

The use of whole blood in expanding the blood volume is, of course, well known but there have been few reports of this having been accompanied by renal function studies. Page (1938) noted the effects of normal transfusions on pregnant women and recorded a slight immediate drop in urine flow in the second hour which is contrary to the results expected from our hypothesis. However his patients were water-loaded to cause a "physiological diabetes insipidus" and if the diuresis were affected through the

posterior pituitary an increased urine flow would be difficult to see on top of a high urine flow.

Blood and plasma were infused into dogs anaesthetized with pentobarbital by Young, Pearce and Stevenson (1955) and changes in renal function followed by clearances of constantly infused creatinine and PAH. A 20% increase in blood volume with plasma always caused a diuresis, accompanied by an unchanged GFR and only a slight increase in the renal plasma flow. Whole blood on the contrary, did not cause a diuresis nor did it increase the right atrial pressure as occurred in the other cases. The GFR, RPF and renal blood flow (RBF) were all decreased below normal. This is rather difficult to explain for whole blood is the most physiological way of expanding the blood volume and should stimulate volume-receptors as well as blood substitutes. It was noticed too, that when a diuresis failed to occur, there was no increase in the right atrial pressure. Why this failed to occur cannot be explained at this time but it seems that blood contains an anti-diuretic factor that plasma for some reason does not.

That anti-diuretic hormone is implicated, is shown by the workers at the Wright Air Development Center (Zuidema, Clarke, Reeves, Gauer and Henry, 1956) who infused dogs (anaesthetized with chloralose) with whole blood, and in 18 cases a diuresis resulted; in 4 there was no change in the urine flow and in 4 there was an oliguria. Two other dogs received transfusions from donors with diabetes insipidus and a diuresis resulted in both cases. They also infused dogs with

normal saline, 6% bovine albumin in .85% saline and canine plasma, all of which produced a diuresis. An oliguria resulted in 13 cases when about 15% of the blood volume was removed by hemorrhage and the urine levels of ADH were high. Anti-diuretic hormone activity was determined by the rat assay method and cases which failed to show a diuresis following transfusion all had high levels of ADH. An explanation for this was offered by Ginsberg and Heller (1951) who suggested that the stability of the anti-diuretic substance was greater in whole blood than in plasma.

Whole blood and plasma infusions are physiological ways of expanding the blood volume to test the hypothesis that volume receptors located in the atria regulate the volume of the extracellular fluid by excreting an increased amount of fluid through the kidneys. Infusions with solutions that are isosmotic and isotonic would not stimulate the osmoreceptors of Verney and a diuresis must be due to some other mechanism such as the one that has been postulated by Gauer and Henry. Considerable doubt has been thrown on this hypothesis and as yet, pathways for the diuretic response to blood volume expansion with blood and plasma are completely unexplained.

Renal Responses to Decreased Stimulation of the "Volume Receptors"

Equally important in the hypothesis that volume receptors regulate blood volume by influencing urine flow, is the effect produced on these receptors by decreases in blood volume or procedures which would tend to collapse the atria. When applied to these cases the hypothesis states that a fall in blood volume would cause less distention of the atria and fewer signals would be sent to the brain by the stretch receptors which would in turn decrease the urine flow. Such methods as dehydration, positive pressure breathing, standing for relatively long periods and other forms of posturing, tourniquets on the limbs and finally hemorrhage would all decrease the effective blood volume in the thorax at least. The work done using these procedures will be summarized below. Salt depletion as a method of decreasing blood volume will not be discussed because it is felt that this introduces too many other variables such as change in tonicity of the extracellular fluid or stimulation of the adrenal cortex.

Dehydration

Although mild dehydration is known to affect the osmoreceptors of Verney, the posterior pituitary can be removed and still some degree of water balance is maintained. Diabetes insipidus dogs excrete large volumes of very dilute urine and yet the glomerular filtration rate remains normal (Shannon, 1942). The oliguria of dehydration which follows in these animals is also accompanied by a hypertonic urine. This ability to concentrate

indicates an active reabsorption of water which in this case cannot be due to the anti-diuretic hormone. This would indicate that another mechanism is operating which would likely be stimulated by the decreased volume and involve tubular reabsorption of solute.

Colloidal Removal of Electrolytes and Water

The extracellular fluid volume can be reduced by another method. Hyperoncotic, isosmotic solutions, if placed in the peritoneal cavity, will remove ECF from the active circulation by dialysis. This was done by Gort (1952, 1954) who injected hyperoncotic albumin and gum acacia in normal saline and observed a reduction in the GFR and RPF and an increase in the chloride and water reabsorption which was in excess of the decreased GFR. From this he believed that the increased colloid osmotic pressure was the stimulus for the observed renal changes. Hyperosmotic dextran was also given intraperitoneally to dogs. This decreased the blood volume and increased the colloid osmotic pressure without changing the tonicity and resulted in a delayed water diuresis and reduced sodium excretion. He concluded the stimulus for the anti-diuresis was the decreased ECF volume.

Mechanical Reduction of Blood Volume

By inflating a balloon in the inferior vena cava, Farber, Becker and Eichna (1953) decreased the venous return to the thorax and produced an anti-diuresis. The stretch receptors

in the atria would record this as a decreased blood volume by sending fewer impulses to the brain.

Fenn, et al. (1947) showed that positive pressure breathing displaced blood to the lower end of the body and hence possibly reduced stimulation of the atrial stretch receptors. Gauer, Henry, Sieker and Wendt (1951, 1954) observed an anti-diuresis with positive pressure breathing during their studies with negative pressure breathing. The effects of positive pressure breathing were noted by Drury, Henry and Goodman (1947). Besides an anti-diuresis, a decrease in the urea clearance was observed which continued for one to two hours after the positive pressure breathing had stopped.

Maintenance of the erect posture has been found to decrease the effective blood volume by causing passive venous congestion of the legs. Brun, Knudsen and Raaschou (1945a, 1945b, 1945c) studied the effect of passive erect posture on kidney function. They accounted for the fall in urine flow in two ways. Afferent arteriolar constriction was responsible for the decreased GFR and RPF and part of the anti-diuresis was due to increased tubular reabsorption of water. Epstein, Goodyear, Lawrason and Relman (1951) extended these studies on the anti-diuresis of quiet standing and found an increased reabsorption of sodium as well as water that was not prevented by an infusion of isosmotic albumin solution. They too, found a decreased GFR which they felt to be partly responsible for the anti-diuresis. They stressed that not only the volume but also the distribution of the extracellular fluid is important.

Another method of decreasing the effective blood volume is by the application of tourniquets which cause a venous congestion of the limbs. Judson, Hatcher, Halperin and Wilkins (1952) using this method, produced an anti-diuresis and a decrease in sodium excretion in normal patients which could be prevented by a large transfusion of albumin solution. This work was extended by Wilkins, Tinsley, Culbertson, Burrows, Judson and Burnett (1953) who observed a decreased RPF, GFR and sodium chloride excretion following application of occluding cuffs to the limbs and suggested that these decreases were part of the general homeostatic mechanism for counteracting the hypotensive effects of an inadequate circulating volume. Splanchnicectomy showed that the mechanism was not dependent on the sympathetic nervous system and the effects were presumably mediated through the neuro-hypophyseal anti-diuretic system, the adrenal cortex and local renal mechanisms. Fitzhugh, McWhorter, Estes, Warren and Merrill (1953) using similar methods, almost duplicated the results except that they found the GFR stable.

Hemorrhage

Hemorrhage, if it is done slowly enough so as not to stimulate the baroreceptors, is a simple and effective way of reducing the blood volume. Lombardo and Viar (1950) and Lombardo, Eisenberg, Oliver, Viar, Eddleman and Harrison (1951) studied the effects of bleeding on electrolyte excretion and glomerular filtration. Small hemorrhages caused a decrease in sodium excretion but no change in the cardiac output or the GFR.

Bleeding larger volumes, (9 ml./kg.) from recumbent subjects caused a striking decline in the sodium excretion and a temporary decline in the GFR that was not prevented by a cuff around the neck. The urine volume was decreased for one hour following the bleeding and it is interesting to note that reinfusion of the blood had no effect.

Hemorrhage causes a decrease in the size of the heart as well as a decrease in the density of the lung (Henry and Gauer, 1951) which would seem to suggest that decreasing the blood volume affects the atrial stretch receptors in the opposite way that blood volume expansion does. If the heart decreases in size, it is likely that the atrial stretch receptors are stimulated less.

Renal function was studied by Corcoran and Page (1943) in nembutalized dogs following bleeding. The diodrast clearance and the inulin clearance fell 25%. Hypotension was found to greatly decrease the inulin extraction. Natravisesh and White (1950) found that bleeding decreases the GFR and RPF and the sodium output but they rose to normal in 1 to 2 days.

More recently, Zuidema et al. (1956) reduced the blood volume in dogs by 15% by bleeding and noted a 50% drop in urine flow in all 13 dogs. They also recorded high levels of anti-diuretic hormone in the urine of these animals which they presumed was the cause of the anti-diuresis.

Bleeding or reducing the blood volume causes a consistent anti-diuresis which is not due to a vasoconstriction if the bleeding is done slowly enough not to cause a fall in blood pressure which would affect the baroreceptors. Also it seems to be a fairly consistent observation that the GFR and the excretion of sodium fall. Although this could account in part for the anti-diuresis, it is likely that the volume receptors are depressed as well. The observations of the decreased size of the heart would tend to support this and indicate that the hypothesized atrial volume regulating reflex could play at least some part in conserving the blood volume.

Possible Effector Mechanisms Controlling Urine Output in Response to Blood Volume Changes

After the centers in the brain have received information of the state of the blood volume from various sources, there are several ways in which the kidney, as the principle effector organ, could be signalled to excrete more or less water and sodium. The efferent pathway could be a hormone, secreted in the brain, or a nervous impulse exerting a direct effect on the renal tubules. Or perhaps there is an intermediary organ which exerts its effect on the kidney. Another possibility is a mechanism which changes the hemodynamics of the kidney.

Role of the Pituitary

Since the discovery of the anti-diuretic hormone and Verney's demonstration that a change in tonicity of the blood is a stimulus for its secretion, it immediately becomes suspect for having another role; that of controlling blood volume. Among other substances, Verney (1947) injected isosmotic saline into the carotid artery but found that it had no effect on the urine flow. If the supra-optico-hypophyseal system is inhibited by an increased blood volume it is not affected by small local expansions in the region of the supra-optic nucleus, such as might be expected to result from these injections of Verney. More recently, Zuidema, Clarke and Minton (1956) studied the effects on urine flow of small intra-carotid injections of solutions of varying osmotic pressure. Ten ml. of isotonic saline in dogs anaesthetized with chloralose, caused no change in the urine flow, whereas hypertonic solutions caused an anti-diuresis.

Although these experiments seem to preclude the possibility of the supra-optico-hypophyseal system reacting to small local changes in blood volume, it is still possible that it could be inhibited through some other sensory mechanism. Recently, Cole (1957) infused hypo-, iso- and hypertonic saline into rats and concluded from studying the sodium excretion that ADH played a role and was sensitive to changes in blood volume.

The possibility of a diuretic hormone being secreted must not be overlooked. Such a hormone has not been isolated but there are suggestions that one exists. The polyuria of

diabetes insipidus can be reduced by removal of the anterior pituitary. Some workers have suggested that the growth hormone may be responsible for this. Shannon (1942) remarked that diabetes insipidus dogs are still able to concentrate their urine despite the fact that the GFR did not change. The effect of growth hormone in increasing PAH and inulin clearances was demonstrated by White, Heinbecker and Rolf (1944). They found that the adrenal hormones were necessary for this action.

Homer Smith summarized the evidence and concluded that the absence of the anterior pituitary diminishes the severity of the polyuria but the gland is not necessary for the production of diabetes insipidus. The anterior lobe probably secretes a renotrophic factor, in the absence of which the GFR, RPF and tubular excretory function are markedly reduced. It does not appear (he says) that the anterior lobe secretes a hormone which is diuretic in effect or directly opposes the anti-diuretic hormone.

Role of the Nervous System

The possibility that tubular reabsorption is under the control of the nervous system was advanced by Cort (1955). He believes that efferent nervous control is manifested directly at the renal tubular cell and modulates the reabsorption of water and electrolytes. He presents evidence for this by stating that denervation of the kidney leaves it unable to reabsorb sodium. Efferent action potentials of renal nerves were recorded simultaneously

with the analysis of urinary excretion. He claims that central connections are in the region of the posterior nucleus of the hypothalamus and the efferent outflow is in the reticular substance of the midbrain and medulla. Lesions in this region (Keeler, 1955) produce a water and salt loss with dehydration which has nothing to do with dysfunction of the endocrine or vascular control. Keeler produced lesions near the paraventricular nucleii of the hypothalamus in rats, which resulted in a large increase in the sodium output but no change in the GFR; diabetes insipidus and ACTH had no effect on sodium output in these animals. Creatinine excretion increased 50 percent in the lesioned rats. The rats returned to positive sodium balance in 3 to 4 days. Depletion of the ECF volume by peritoneal dialysis was followed by a 60 percent reduction in sodium excretion. It was concluded that the volume of the ECF was the stimulating mechanism for sodium excretion and that this was mediated through nervous pathways.

Opinions regarding the role of the renal nerves are widely divergent mostly because observations have been made under widely varying conditions. Some are acute preparations, others chronic, anaesthetized and unanaesthetized, hydropenic and hydrated. Renal denervation in itself is technically difficult and it has been said that the only way to be sure that it is complete is to transplant the kidney. It is therefore not surprising that opinions are so widely divergent. There seem to be two schools of thought. One group found a polyuria and

an increased chloride excretion with renal section (Marshall and Kolls, 1919). Page et al. (1954) noted that unilateral splanchnic nerve resection is accompanied by an increased sodium loading. The urine flow and sodium excretion increased after renal denervation according to Sartorius and Burlington (1956). Surtshin and Schmandt (1956) observed a similar phenomenon but the increase in sodium excretion got less with time (15 days). It was suggested that this could be explained by increases in the GFR. Study and Shipley (1950) stimulated the renal nerves electrically which resulted in a reduced urine flow, RPF and GFR.

Of the opposite opinion are those workers that believe that the renal nerves have no effect on renal function at all. Berne (1952) using dogs that had had the left kidney denervated 6-49 days previously, came to the conclusion that differences in renal excretion of sodium and water could be explained by the fact that the animals that did show a difference were under anaesthesia while those that showed no change were unanaesthetized. One explanation seems to be that under anaesthesia, enhanced vasoconstrictor influences cause a reduction in the GFR in the kidney with intact innervation.

After reviewing the literature, Homer Smith has concluded that denervation has no effect on the autonomy of the renal circulation, normal urine flow, water diuresis or pituitary anti-diuresis. However, it seems safe to say that at this stage with so many conflicting opinions, no definite conclusion can be drawn.

The Influence of Hemodynamic Factors

A third way in which the kidney could excrete more fluid and electrolytes is by increasing the rate at which the blood is filtered by the glomeruli. This would present an additional load to the tubules which would make reabsorption more difficult. There seems to be some correlation between arterial blood pressure, venous pressure and water and electrolyte output.

The Effect of Venous Pressure Increases

Selkurt (1954) has reviewed the literature on the effect of venous pressure on sodium excretion and has presented his own conclusions that small acute changes in the GFR, too small to be detected by present methods may account for the observed alterations in total sodium output.

In order to find more proof for the theory that atrial stretch receptors are volume receptors, Gauer, Henry and Sieker (1956) gave infusions of 400-600 cc. of blood to normal males or bled them a similar amount. The rise in central venous pressure corresponded closely with the time of infusion and they concluded on this rather scanty evidence that the low pressure system acts like a distensible container and suggested that the changes in volume and pressure may serve as a mechanism for blood volume control. Henry, Gauer and Sieker (1956) using dogs anaesthetized with morphine and chloralose, bled or infused them with blood up to 40 percent of their blood volume, and found

little change in the systemic pressures although the right and left atrial pressures seemed to reflect changes in the blood volume. If the atria were the volume receptors, they suggested that they could be effectively stimulated by changes in blood volume.

The Effect of Systemic Arterial Pressure Increases

The "autonomy of the renal circulation" has been a well-known phenomenon for many years. Despite variations in systemic blood pressure the renal circulation remains surprisingly constant. Pappenheimer and Kinter (1956) have offered one explanation for this in their "cell-separation" theory. Briefly, this postulates that a cell-rich moiety of blood short-circuits the tubular circulation because plasma skimming separates a plasma-rich fraction into a long peritubular route. This allows autoregulation of the renal blood flow because the amount of plasma skimming is dependent on the pressure and the viscosity of the blood. At low pressures, there would be less skimming and more filtration of the outer glomeruli.

Selkurt (1949, 1951, 1946) has investigated the problem of the effect of systemic arterial pressure on GFR and total renal blood flow. He found that there was considerable autonomous control of the renal blood flow but speculated that small changes in the GFR, undetectable by present methods, may account for an increase in the sodium and water excretion.

The answers to these questions are still undecided but most workers agree that the renal circulation is autonomously controlled in some manner at systemic pressures above 60 mm. Hg. Although changes in the GFR may contribute to the urinary responses observed following isotonic infusions, it seems apparent that a search must be made elsewhere for the cause of both the diuresis and small GFR changes than the effect of minor fluctuations in systemic blood pressure on renal blood flow.

Other Possible Mechanisms Involved in the Control of Blood Volume

Besides the factors which control the amount of fluid and electrolyte intake such as thirst, there are several other factors which could have a hand in controlling blood volume. Little is known about some of these, such as the effect of increases in cardiac output and interstitial pressure and they will not be discussed further.

Most important is the regulation of sodium excretion because sodium, as the chief electrolyte, could control indirectly the amount of water excreted. Since sodium concentration is known to be regulated by the adrenal hormones, a study of the corticoids becomes very important. Gaunt, et al. (1955) state that the effect of the corticoids on water excretion is probably dependent on some interplay between the following

factors: (1) their effect on sodium excretion; sodium retention being an anti-diuretic influence and sodium loss a diuretic influence; (2) their effect on the glomerular filtration rate; and (3) their effect on the tubular reabsorption of water, which may be direct or conditioned by concurrent activity of the neurohypophysis. The result is that corticoids may be diuretic or anti-diuretic, depending on the nature of the steroid used, and the dosage as well as the physiologic conditions under which they have to work.

Regulation of Sodium Excretion as Related to the Control of Blood Volume

The effects of the mineralocorticoids are well known from their sodium-retaining properties. In adrenal insufficiency, there is an increased elimination of water as well as a loss of sodium. Since the crystallization of a new potent sodium-retaining hormone, aldosterone, from the amorphous fraction of adrenal extracts by Simpson and Tait in 1952, there has been an increased interest in the use of hormones to control blood volume. This substance has 15-30 times the sodium-retaining properties of DCA.

The first evidence that adrenal corticoids had any effect on urine volume was disappointing because Desaulles, et al. (unpublished) found that aldosterone had no effect on water excretion at any dosage level. DCA on the other hand was found to be anti-diuretic in proportion to the dosage. In patients with primary aldosteronism (Conn and Louis, 1956) no consistent relationship could be found between the urine volume and urinary

titers of the hormone. These patients did not have edema. It is noteworthy that Rosenbaum, Papper and Ashley (1952) found that patients with Addison's disease could still excrete a normal water load with hypotonic saline despite the lack of sodium excretory response. Leaf and Mamby (1952) on the other hand, found that patients with Addison's disease could not excrete a water load unless they were maintained with cortisone as well as DCA. They found that ADH levels were very high despite a hypotonic plasma and suggested that the stimulus for the hormone was a decrease in the ECF volume. Gaunt (1955) found however that aldosterone caused a distinct increase in water diuresis when given to adrenalectomized rats hydrated with distilled water per os. Its diuretic potency equalled that of hydrocortisone and exceeded that of cortisone or DCA. These reports are so conflicting that it appears to be too early to draw any helpful conclusions.

Farrell, et al. (1954) found that hypophysectomy in the dog did not affect the secretion of aldosterone. The fact that aldosterone secretion continues in the absence of the pituitary and ACTH suggests that it may have functions different from the other corticoids.

Since sodium retention is known to occur in congestive heart failure, together with the fact that aldosterone levels are also high, lead several workers to hypothesize that aldosterone may be a factor in controlling blood volume. Artificial right

heart failure and ascites were produced in dogs by constriction of the inferior vena cava and pulmonary artery (Davis, et al., 1956). Bioassays of urinary aldosterone showed an increased activity and a sodium retention. After adrenalectomy, there was a fall in the aldosterone index with an increased sodium excretion.

The effect of a reduced blood volume on plasma aldosterone levels was demonstrated by Farrell, Rosnagle and Rauschkolb (1956) who bled dogs about 30 ml./kgm. Large samples of adrenal venous blood were necessary for the determinations and although some blood replacement was attempted with dextran in normal saline, the mean arterial blood pressure fell steadily. Aldosterone levels rose although no change in electrolyte concentration occurred.

Patients with edema of cirrhosis and congestive heart failure were studied by Duncan, Liddle and Bartter (1956). In normal men urinary aldosterone levels fell when intravenous normal saline was given. When the same infusion was given to edematous men, the reduction of urinary aldosterone levels was less. They felt that the failure of the aldosterone levels to fall was a factor in the formation of the edema.

The same group (Bartter, Liddle, Duncan, Barber and Delea, 1956) expanded the body fluids by giving pitressin and they found a decrease in urinary aldosterone and a hyponatremia. They concluded that decreases in aldosterone secretion were produced by measures which increased ECF volume. They state that some function of the volume increase serves to inhibit

secretion. They could not suggest however what this function was nor how information was transmitted to the brain. They do not believe that this is the only mechanism for controlling blood volume.

In conclusion it appears that the adrenal corticoids, especially aldosterone, respond to changes in blood volume. No one seems to think that this is the only mechanism for controlling blood volume but rather that it is only a single mechanism supporting other more complicated ones.

Other than the suggestion of Cort that there is nervous control of tubular reabsorption of sodium, there appears to be little known about either mechanisms or pathways by which such a control of blood volume might be effected. It seems likely that the answer lies, not in a single mechanism but in a combination of many. As Homer Smith summarizes, "by physiologically integrating the renal mechanisms controlling the excretion of sodium, the supra-optico-hypophyseal mechanism controlling the excretion of water, the glomerular apparatus controlling the filtration rate and the action of the hormones of the adrenal cortex and neurohypophysis on the distal (?) system, one would have a system that would regulate not only the composition of the extracellular fluid but its volume as well."

Objectives and Reasons for the Investigation

The objectives of this investigation are in general to acquire more information about the afferent and efferent pathways of a mechanism controlling blood volume. An hypothesis has been advanced (Henry and Gauer) that atrial stretch-receptors and the vagus nerves form the afferent limb of a reflex mechanism controlling blood volume. Although there seems to be adequate evidence that vagal fibers carry impulses to the hypothalamus from the atria in response to stretch, evidence is lacking that this sensory arrangement has control over the regulation of blood volume. Stimulation of the atrial receptors by methods that are rather unphysiological (negative pressure breathing, etc.) indicate that these receptors influence urine flow but information on the mechanism using physiological methods of expanding the blood volume is scanty. It is one of the purposes of this investigation to gain more information about renal responses to hypervolaemia by the use of a natural physiological blood volume expander, plasma.

Since there is some doubt that the atrial receptors play a part in controlling blood volume, the second purpose of this investigation is to section the afferent pathways, that is, the vagi and to note the effects of this procedure on the responses to increased blood volume.

Thirdly, an attempt was made to secure information on the effector mechanisms of a reflex that controls water and electrolyte excretion in response to changes in the intravascular volume. There are very few reports of this type of investigation in the literature. In addition, studies of urinary sodium excretion were undertaken in the hope of securing information as to the relation of this to blood volume control.

Finally, a comparison of the effects of bovine albumin solution as a blood volume expander will be compared with plasma in order that information gained here may be compared more advantageously with work already done.

An Outline of the Reasons for the Experimental Approach Used

The experimental animal used was the dog because most work on this subject has been done using this animal and it is of convenient size for the procedures necessary. The experiments were conducted under surgical anaesthesia for facilitation and because acute surgical procedures could be more easily carried out. Plasma was used as the blood volume expander because it is easily acquired and dangers of allergic reactions are minimized.

Renal function tests were done using exogenous creatinine and p-aminohippurate in order to reveal changes in renal dynamics that might occur during alterations in urine output. A constant level of exogenous creatinine was maintained as this was felt to be the most accurate method of obtaining renal clearances.

It was felt that the most effective way to study the effector mechanisms of this reflex would be to remove an organ that would likely control urine output. Because of the many interesting reports recently appearing in the literature concerning aldosterone and its secretion in hypervolaemia, it was felt that information following removal of the adrenals would be especially pertinent. The adrenalectomized dogs received replacement therapy to keep the body levels of adrenal corticoids stable and when they had recovered from the operation, their response to an increased blood volume was observed. The operative procedure was relatively simple and the animals were easily maintained.

This brief review of the literature has shown that there are several inconsistencies in the experimental evidence which conflict with the present hypothesis on blood volume control. Most of the conflicting evidence is concerned with the various kinds of diuretic responses to different infusions and the persistence or absence of the diuretic response after vagotomy. There is almost no information about the effector mechanisms of this diuresis. It is on these problems that some information is hoped to be obtained.

The problem may not only yield fundamental information about an important and puzzling homeostatic mechanism but may also shed light on the problem of sodium and water retention found in patients with cardiac insufficiency.

METHODS

Preparation of the Experimental Animal

Mongrel dogs of both sexes, weighing 5 - 15 kgm. were used. No extra water or salt was given them on the assumption that dogs will regulate their intake according to their needs. Water was given ad libitum. Their food consisted of a balanced dog chow preparation. In a few of the later animals, a small intravenous infusion of Ringer-Locke solution was given in order to increase their basal sodium output.

Surgical anaesthesia was produced by intravenous administration of sodium pentobarbital (Nembutal) 30 mgm./kgm. Polythene cannulae filled with dilute Heparin solution were inserted into both femoral veins to a level where the tips were judged to be at the lower end of the inferior vena cava. One femoral artery was also cannulated to a similar level. The right jugular vein was also cannulated with a large polythene tube to the right atrium. From a midline incision in the neck, both vagus nerves were exposed, two cotton ligatures tied loosely around each and then replaced to their positions in the neck with a minimum of trauma. A Magill intratracheal tube was sometimes inserted through the mouth if the animal showed any signs of respiratory difficulty.

Cannulation of both ureters was done through a single incision in the left flank. A muscle-splitting incision

was used to expose the peritoneum. The left ureter was usually found lying at the bottom of the incision. By gently dissecting through the loose connective tissue separating the peritoneum from the posterior wall, the right ureter could be visualized. Both were then cannulated with fine polythene tubing and the tubing brought out through the incision. Care was taken not to tug on the ureters as this was felt to be one of the causes of the hematuria which sometimes occurred. (See appendix for a more detailed account of the operative procedure.) Urine was collected in 10 ml. graduated cylinders.

Particular care was taken to tie off all the small bleeding vessels because these could result in considerable blood loss after the animal was heparinized when they became very difficult to locate.

One-half hour was allowed for the animal to recover from the operation and then 2 mgm./kgm. of intravenous heparin was given. The arterial cannula was attached to a mercury manometer to record arterial blood pressure. The jugular cannula was attached to a water manometer which had previously been zeroed to approximately the level of the right atrium. The entire procedure from the time of anaesthetization to the completion of the operation, took about 1 1/2 hours.

Anaesthetic

Chloralose was used in the preliminary experiments because it had been found by Homer Smith (also Theobald, 1934;

Newton and Smirk, 1934; Vincent and Thompson, 1928) to be the only anaesthetic that did not inhibit a water diuresis. Also, much of the previous work (Henry and Gauer) had been done using this anaesthetic. We found chloralose unsatisfactory and discarded it early in the series. It was found to have the following disadvantages:

(1) Difficulty of administration. It is very insoluble and has to be kept at 60 degrees F. Large doses at very frequent intervals are necessary.

(2) Convulsive twitchings often resulted from its administration.

(3) The only material which could be obtained here was not biologically standardized.

(4) Lower than normal urine flows resulted from its use.

(5) There were indications that the urine flow varied with the depth of anaesthesia.

(6) There was great difficulty in keeping the animal at a constant depth of anaesthesia.

Sodium pentobarbital was found to be much more satisfactory. The basal urine flowwas more nearly normal and a constant level of anaesthesia was maintained by putting Nembutal in the slow constant-infusion bottle used for maintaining plasma levels of Creatinine and PAH. Corcoran and Page (1943) have shown that it does not significantly affect renal function.

Renal Clearances

Concept of Renal Clearances

Creatinine has the property of being completely filtered by the glomeruli in its passage through the kidney and is not absorbed or excreted by the tubules. Therefore, the amount found in the urine is related to the glomerular filtration rate or the amount of plasma water passing through the glomeruli in a given moment. The calculation is completed by dividing the urine output by the plasma concentration $C_{cr} = \frac{uv}{p}$.

The clearance of p-aminohippurate (PAH) can be used as an indication of the amount of plasma flowing through the kidneys in a given moment. It has the property of being almost completely cleared from the blood in its passage through the kidney. It is filtered by the glomeruli but as well it is excreted by the tubules. Hence the amount found in the urine is an indication of the amount of plasma flowing through the kidney if this is divided by the plasma concentration.

Unfortunately in the dog, PAH enters the red blood cells and 5 percent passes through non-functioning renal tissue so it cannot be completely cleared from the blood. An extraction ratio of 85 percent is considered normal for the dog. If allowance is made for this, the renal clearance of PAH is equivalent to the renal plasma flow or more accurately, the effective renal plasma flow, for this does not include blood which may bypass the glomeruli and tubules through shunts. (Truetta, Pappenheimer and Kinter, 1956).

Measurement of Renal Clearance

A priming dose of creatinine and PAH (see later) was given shortly after completion of the operative procedure. It was dissolved in a minimum of distilled water and injected intravenously. Then a constant-drip infusion of a solution containing creatinine and PAH in a concentration equal to their excretion was set up. The infusion was maintained at about 1/4 to 1/2 cc./min. and also contained 2 to 3 mgm. of heparin and an anaesthetic dose of sodium pentobarbital. Forty to fifty minutes were allowed for the equilibration of the plasma levels to the test substances before commencement of any clearance periods.

Urine collections were made over a 10 to 15 minute period and in the middle of the period 6 to 7 ml. of venous blood was withdrawn and immediately centrifuged at 2500 r.p.m. for 15 minutes, and the plasma pipetted off into a clean test tube. A drop of heparin was usually added to the centrifuge tubes to ensure against clotting. Samples were taken for at least two consecutive clearance periods in every case, for every interval in which renal function measurements were desired. In later experiments it was found that since the plasma levels of creatinine and PAH remained so steady, only one blood sample was necessary for every pair of urine samples. This reduced the amount of blood taken from the animal and delayed the inevitable deterioration in the animal's condition.

Because of the fine tubing used for cannulating the ureters, the dead space involved in urine volume measurements was negligible and was not accounted for.

Maintenance of Creatinine and Plasma PAH Levels

To avoid errors inherent in the single-injection technique, plasma levels of creatinine and PAH were maintained by a constant slow infusion following the priming dose. The infusion was maintained by forcing the fluid under pressure through a fine capillary tube. The rate of flow could be adjusted by varying the pressure in the bottle.

The priming injection was calculated as follows:

$$\text{Priming dose (mgm.)} = \underset{\text{(kgm.)}}{\text{Wt. of animal}} \times \frac{\text{Vol. of distrib.}}{100} \times \text{intended plasma conc. (mgm./ml.)} \times 1000$$

The volume of distribution for creatinine is 50 percent and for PAH is 30 percent of total body weight (Greenberg, et al., 1952). Homer Smith has shown that if the plasma concentration is maintained between 7 and 15 mgm. percent the error due to endogenous chromagens is reduced to insignificant levels. An attempt was made to keep the plasma level of PAH between 1 and 2 mgm. percent because Homer Smith has shown that a concentration above 4 mgm. percent does not allow complete clearance to take place.

The sustaining intravenous infusion was calculated as follows:

$$\text{Conc. of sustaining infusion in mgm./ml.} = \frac{\text{plasma conc. (mgm./ml.)} \times \text{estimated clearance (ml./min.)}}{\text{rate of infusion (ml./min.)}}$$

The estimated clearances for creatinine (4.29 ml./kgm./min.) and PAH (13.5 ml./kgm./min.) were based on values quoted by Smith (1951). The rate of infusion was about 0.5 ml./min. or less; a rate which would have little effect on the animal's blood volume.

Preparation and Infusion of the Blood Volume Expander

Plasma, either fresh or stored at 5 degrees C. was used. Stored plasma was usually taken from the dog of the previous experiment and stored with additional heparin. Blood was withdrawn from the donor animal via a catheter in the femoral artery. The flow was regulated to a slop drip and continued until complete exsanguination. The blood was centrifuged at 2000 r.p.m. for 45 minutes; the plasma withdrawn and recentrifuged. Some degree of hemolysis always resulted despite careful handling.

In every case when the plasma was stored, a filmy gelatinous precipitate formed which we assumed to be fibrin. When dried and weighed, it was found to be only a negligible portion of the total plasma proteins, so plasma was only filtered for use.

Some recent work (Smith and von Korff, 1957) has shown that this is likely to be a fibrinogen fraction which combines with heparin, precipitates and coagulates in the cold. Since the plasma proteins are responsible for only a small fraction of the osmotic pressure of the plasma, it was felt that the loss of this small fraction of the proteins would have a negligible effect on the tonicity of the plasma.

Before use, the plasma was warmed to 39 degrees C. and infused by a gravity-drip system. The infusion rate was approximately 1 cc./kgm./min. and generally took 15 to 20 minutes to complete. The infusion sometimes brought the blood pressure up to normal but seldom higher.

In the experiments where bovine albumin was used 6 percent by weight of the powder was mixed in Ringer-Locke solution made with de-ionized water. It was dissolved with the aid of a constant-temperature bath and filtered ready for use.

The amount of the blood volume expander used was 15 to 20 percent of the blood volume which was calculated as 8.5 percent of the animal's weight in kilograms. Rarely, a larger amount was necessary in order to produce a diuresis.

In the last five experiments that were done, an antihistamine (chlor-tripolon), 0.5 mgm./kgm., was added to the infusate to eliminate the possibility of histamine being the cause of the diuresis.

Chemical Procedures

Determination of the Plasma Creatinine

The creatinine concentration was determined by a modification of the Folin-Wu alkaline picrate method (Bonsnes and Taussky, 1945). Varley's "Practical Clinical Biochemistry" was found to be very helpful. A protein-free filtrate was obtained by adding 1 ml. of plasma to 8 ml. of water and then adding 0.5 ml. of 10% sodium tungstate and 0.5 ml. of 2/3N-sulfuric acid. This was thoroughly mixed and allowed to stand for five minutes before being filtered. It is important to shake thoroughly to mix.

To 3 ml. of the filtrate in a 5 ml. Fisher electrophotometer tube, 1 ml. of .04M picric acid was added, plus 1 ml. of .75N- NaOH. The optical density of the red-colored tautomer of picric acid was read in a Fisher Electrophotometer against a water blank set at zero optical density. Duplicate determinations were always done.

From the average of the duplicates the concentration in mgm. percent was read from a standard curve plotted on squared paper showing optical density against concentration in mgm./100 ml. of plasma.

A standard curve was plotted every time fresh reagents were prepared, using fresh plasma diluted in water which had been replaced with from 1 to 8 ml. of a standard solution containing 4.00 mgm. percent of creatinine and 1.00 mgm. percent of PAH.

Determination of Urine Creatinine

One ml. of urine, or 0.5 ml., if the 10 minute volume was under 3 ml., (or 2.0 ml. of urine if the 10 minute volume was over 10 ml.) was pipetted into a 100 ml. volumetric flask and brought up to the mark with distilled water. One ml. of this was added to 10 ml. of water in a test tube and 3 ml. of the resultant mixture was pipetted into a Fisher electrophotometer tube. Picric acid and sodium hydroxide were added as before and the mixture then read in the electrophotometer.

Optical density readings were converted to concentration in mgm. percent from a calibration curve made from various dilutions of a standard solution containing 1.25 mgm. of creatinine and .75 mgm. PAH/ml.

Determination of Plasma PAH

This method is a modification of the Smith, Finkelstein, Aliminosa, Crawford and Graber (1945) method which is a modification of the method for the determination of sulfonimides. Cadmium sulfate was used to precipitate the proteins (Fujita and Iwatake, 1931).

One ml. of plasma was added to 10 ml. of distilled water and then 3 ml. of acid Cadmium sulfate was added, followed by 1 ml. of 1.1N sodium hydroxide. (Acid Cadmium sulfate was prepared by dissolving 17.34 gms. of Cadmium sulfate ($3CdSO_4.8H_2O$) in 84.55 ml. of N- sulfuric acid and made up to 500 ml.) After a thorough shaking the mixture was filtered. To 2 ml. of filtrate in a Fisher electrophotometer tube, 0.4 ml. of 1.2N- hydrochloric acid and 0.2 ml. of 0.1% sodium nitrite were added and mixed well. After

standing for five minutes 0.2 ml. of 0.5% ammonium sulfamate was added to remove the excess nitrite. After mixing it was allowed to stand for another three minutes and then 0.2 ml. of the coupling reagent N-(1-naphthyl) ethylenediamine dihydrochloride was added. This was mixed by inversion and ten minutes was allowed for the blue color to develop. It was read in the electrophotometer against a water blank. It was extremely important to mix the ingredients between each step or a false dark color would develop.

The reagents are prepared in the concentrations stated above. Sodium nitrite was made fresh daily and ammonium sulfamate was made fresh every two weeks. It is necessary to keep N-(1-naphthyl) ethylenediamine dihydrochloride cold and away from light.

A calibration curve was plotted from the same plasma standard as was used for the creatinine but appropriate dilutions were made.

Determination of Urine PAH

The method is similar to that for plasma except that protein precipitation is not necessary. One ml. of urine was diluted to 100 ml. with distilled water. If the urine was very dilute, 2 ml. of urine were often used. One ml. of this solution was added to 20 ml. of water in a test tube. Two ml. of this were pipetted into a Fisher electrophotometer tube and the color developed in the same way as mentioned above. A

separate calibration curve was made using the urine standard solution.

Hematocrit

The hematocrit was determined for each blood sample by using Wintrobe hematocrit tubes spun at 2200 r.p.m. for 15 minutes. These were inaccurate at times because the blood sometimes tended to clot in the tubes on account of the animal having been given a minimum of heparin. No correction factor was applied for the plasma trapped in the packed cells.

Flame Photometry

By the use of the Beckman DU Flame Spectrophotometer all urine samples were analyzed for sodium content. The urine was diluted 1 to 50 with distilled water. A standard curve was plotted before every set of determinations using various dilutions of an artificial average stock urine (Mosher, et al.,1949) containing Na^+ 135 mEq/liter and K^{+50} mEq/liter and 20 gms. of urea/liter. Successive dilutions of this stock urine were made to give eight points on a standardization curve ranging from 27 mEq/l. to 236 mEq/l. Potassium was added to the stock urine because it is normally found in urine and has been found to accentuate the sodium flame. The working standard solutions were made up as follows: .4, .6, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 ml. of the stock solution were diluted to 100 ml. and stored in plastic bottles for use in making the standardization curve.

Readings of the standards and urine samples were "gross" luminosity and to get the "net" luminosity the reading of the blank or solvent, in this case water, was subtracted from the gross luminosity readings. These values were then transposed to concentration in mEq/l. from the calibration curve. Total concentration of sodium in the urine for each 10-minute sample period was expressed in mEq. and calculated by multiplying the concentration by the urine volume and dividing by 1000.

RESULTS

Analysis of the Experimental Method

Instrumentation Error

(a) Duplicate Samples

To estimate the accuracy of the analytical determinations for creatinine and PAH, using the Fisher electrophotometer, in 30 random pairs of duplicate samples optical density readings were compared. Presumably the duplicates differed only in time and the sum of the differences between the first and the second readings of a large sample should equal zero. Since this was obviously not the case, a statistical analysis was done to find out whether or not the difference between the first and second readings could be accounted for only by chance.

'Students' t test was applied to the difference between the first and the second readings taking the sign into account. First the sample variance was calculated for the 30 discrepancies and then applied to the formula:

$$t = \frac{\left[\bar{X}_o - \bar{x}\right]\sqrt{n-1}}{s}$$

The value for t was applied to Fisher's t tables to find the probability level.

From this it was shown that the difference was large enough to be "highly significant" in the determinations for

---

*When the calculated variation due to chance alone was 5%, the results were considered "significant", if less than 1%, "highly significant".

plasma creatinine, urine creatinine and PAH plasma but not for urine PAH. The second reading was usually greater than the first. The reason for this could not be ascertained but may have been due to drifting of the needle between the two readings. It is therefore suggested that the electrophotometer be zeroed between every reading instead of once for every set of duplicates as was done.

No indication could be found as to which of the duplicates was the more accurate. Hence, it was felt that an average of the two readings was satisfactory in determining the concentration. It will be shown later from the recovery curves that this caused little inaccuracy.

Duplicate readings were not made for the flame photometer because the reproducibility of the individual readings was good (see below) and because the instrument was recalibrated after every tenth reading in order to permit minimum drifting of the needle. It was felt that this technique gave maximum accuracy.

(b) Reproducibility of Readings

The degree to which individual readings could be reproduced was also estimated by taking 10-20 readings on the same sample and subjecting them to statistical analysis.

With the electrophotometer 20 readings of the same sample with zeroing between readings resulted in an average optical density reading of 16.9. The average discrepancy from this mean was 0.1 which is 0.6% variation from the mean. This is within an acceptable range.

Using the Fisher Electrophotometer, concentration of the samples was read as optical density times 100. Samples were diluted so that the optical density was less than 40 but because the scale became smaller as the readings increased, the error became larger with the higher values. This was an inherent characteristic of the machine and could not be overcome.

The flame photometer on the other hand gave excellent reproducibility. Twenty repeat readings of a single sample gave exactly the same result. Because of a slight flicker of the null meter needle, no attempt was made to take readings closer than 0.5% transmission.

Error of the Chemical Determinations

Chemical determinations were done for creatinine and p-aminohippurate (PAH) in plasma and urine. To test the accuracy of the procedure, known amounts of creatinine and PAH were added to water in one case and plasma and urine in another test. The difference in the amount of creatinine and PAH recovered by the two methods would be an indication of the effect, if any, that plasma or urine had on the determinations.

These recovery curves are shown in Fig. 1 and as can be seen, the two curves are always close together. There is a small amount of endogenous creatinine in urine and plasma and this is the reason for these two curves being higher at first than their comparable "water" curves. It will be noticed also that less creatinine and PAH was recovered from plasma than from water and this can be explained by the protein-precipitant which probably took some creatinine and PAH with it.

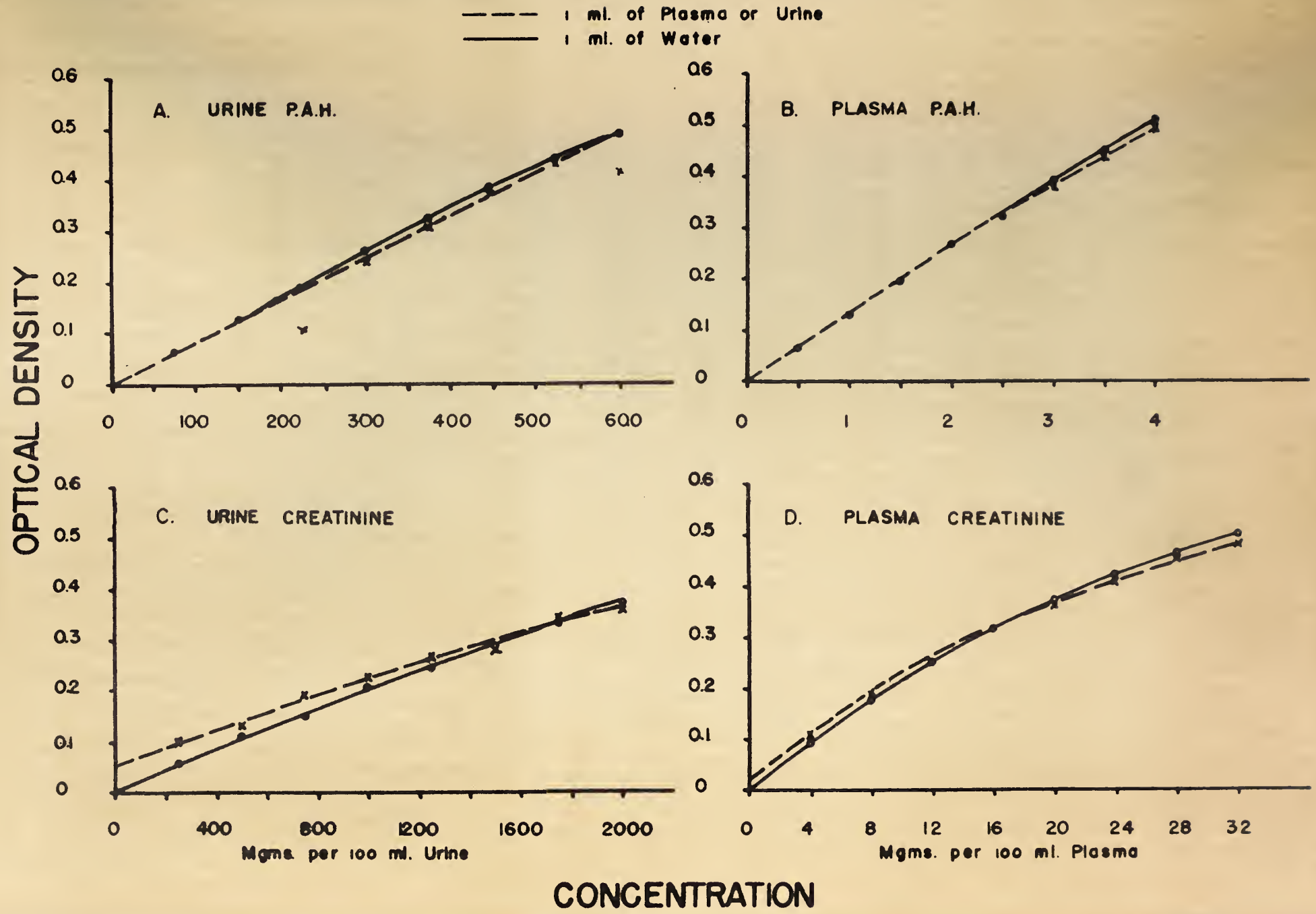
The determinations all gave reasonably smooth curves except in the case of the urinary PAH recovery curve. This was repeated several times and there were always one or two points that fell outside the best curve drawn by eye. This could not be explained because when water was used instead of urine, a smooth curve always resulted. There must be some substance in the urine which occasionally affects the determinations. These may account in part for the greater variation in the calculated renal plasma flows than in other indices that resulted in the experimental series.

In all cases, calibration curves were made using plasma and urine and this was felt to make consistent errors insignificant.

Estimation of sodium concentration in the urine was also carried out on the animals. Before every run, the

FIGURE 1

Graphs showing recovery of known amounts of creatinine and PAH from urine and plasma compared to the recovery from the same amount of water.


1 ml. of Plasma or Urine
1 ml. of Water
A. URINE P.A.H.
B. PLASMA P.A.H.
C. URINE CREATININE
D. PLASMA CREATININE
OPTICAL DENSITY
0.6
0.5
0.4
0.3
0.2
0.1
0
0 100 200 300 400 500 600
0 1 2 3 4
0 400 800 1200 1600 2000
Mgms. per 100 ml. Urine
0 4 8 12 16 20 24 28 32
Mgms. per 100 ml. Plasma
CONCENTRATION

flame photometer was calibrated with diluted standard solutions of varying concentrations. When these points were plotted on squared graph paper, a smooth curve resulted. This curve was very reproducible if the various instrument settings were the same. Since the standards were prepared from an artificial urine containing 135 mEq./l. of sodium and 50 mEq./l. of potassium plus 20 gm. of Urea/l., no other check was felt to be necessary.

In the conclusions, absolute values were less important than changes in concentration, which may justify acceptance of minor calibration irregularities.

Analysis of the Control Experiments

Control Urine Flow

The average control urine flow for seventeen dogs (average weight = 10.6 kgm.) was 0.31 ml./minute for series I and II. Priming the animal with Ringer-Locke solution two hours before the start of the experimental procedure had little effect on the basal urine output. The average urine flow of nine dogs primed with Ringer-Locke solution (10 ml./kgm.) was exactly the same as that of the dogs that were not primed.

Of the five control animals that were done, two showed large spontaneous increases in urine flow lasting from

thirty to eighty minutes. Since these appeared in the middle of a control period, no reason could be found for their occurrence. They were accompanied by an increase in sodium output but no consistent change was found in the concentration of the sodium excreted. The mechanism of these spontaneous diureses could not be ascertained.

The possibility of such spontaneous diureses being confused with those resulting from an increased blood volume was taken into account by altering the blood volume only when the urine flow was stable or decreasing.

Control Sodium Output and Concentration

There was great variation in the amount of sodium in the urine from period to period and from dog to dog. A great deal of difference depended on whether or not the animal had received a priming injection of Ringer-Locke solution. The sodium concentration was about ten times greater in those that had received intravenous saline.

A plot of experimental data is recorded in figures 11 and 12 and it will be noted that the sodium output roughly parallels the urine output. A priming dose of intravenous saline raises and maintains the sodium excretion for the duration of the experiment.

Control Glomerular Filtration Rate

The average value for all the control glomerular filtration rate (GFR) determinations done on 22 dogs was 4.40 ml./kgm./min. This compared favorably with the value of 4.29 ml./kgm./min. quoted by Smith (1951).

Considerable variation was found in the GFR's of individual animals. Of the four control animals the standard deviation was ± 0.6. Control dog C-4 (see Fig. 12) had an average GFR of 5.17 ml./kgm./min. with a standard deviation of ± 0.57.

A significant difference was shown to exist between the values for individual dogs. An analysis of variation of the GFR's was obtained for the four control dogs. The variance ratio was determined and, using Snedecor's table of F, a "highly significant" difference was found to exist. ~~even at the 1% level~~. This made it impossible to compare values for different dogs. Any change in the GFR could only be determined statistically by comparing values obtained with those of a short control period. The control period was found to be too short and a statistical analysis could not be done.

However, to show the variation that occurred normally and the limits of normal variation, the average deviation from the mean was determined and represented as a percentage of the mean. This was found to be ± 10 percent. It is realized that

this is only a rough representation of the variation but when plotted on the scatter diagrams as will be shown later, it gives a useful index of the range of variation and the total measurement error.

Control Renal Plasma Flow

The change in the renal plasma flow (RPF) from moment to moment was great but the average RPF control values for 22 dogs was 12.25 ml./kgm./min. Smith (1951) quoted a value of 13.5 ml./kgm./min. as an average. This difference is acceptable if it is considered that there was considerable variation between successive readings. Control dog C-4 (see fig. 12) had an average RPF of 15.38 ml./kgm./min. and a standard deviation of ± 1.89 which gives an example of the variation.

All control values for the RPF were treated in the same manner as the GFR's. The average deviation from the mean was 10.9% and this was plotted on the scatter diagrams which follow.

Despite the large variability of the RPF determinations there was no reason to believe that this was due to technical irregularities. It was probably due to the wide biological variations and the conditions of the experiment.

Effect of Vagotomy

Of nine experiments in which vagotomy was done before any other procedure, a definite increase in urine flow followed in five of these. The diuresis was accompanied by a naturesis and the sodium concentration changed little. No consistent change was found in the GFR or RPF after vagotomy.

Analysis of the Experimental Animals

The experimental work was divided into three series. Series I consisted of 14 dogs in which plasma was used as the blood volume expander. Series II consisted of 4 dogs infused with 6 percent bovine albumin in Ringer-Locke solution as the blood expander. Series III was one in which the blood volume of adrenalectomized dogs was expanded with plasma. This consisted of 6 dogs and will be discussed separately. The effects of an increased blood volume before and after vagotomy on urine flow, GFR, RPF and sodium output will be summarized.

Urine Flow

In both series I and II a diuresis almost invariably followed expansion of the blood volume regardless of whether or not vagotomy had been performed previously. In series I the average diuretic urine flow was 4.8 times the control value before vagotomy and 3.6 times the control value after vagotomy (Table I). The average urine output during the diuresis in

Table I. Average urine flows and sodium concentrations during the diuresis compared to average control levels.

Dog	URINE FLOW					SODIUM CONCENTRATION					
	Control average	Pre-Vagotomy Average	Pre-Vagotomy Peak	Post-Vagotomy Average	Post-Vagotomy Peak	Control immed. before infusion	Pre-Vagotomy At start diuresis	Pre-Vagotomy At peak diuresis	Control immed. before infusion	Post-Vagotomy At start diuresis	Post-Vagotomy At peak diuresis
Series I											
A-18	0.26	.59	.77	-	-	90	140	190	-	-	-
A-19	0.13	1.4	2.2	.53	.55	224	240	185	110	84	100
A-21	0.3	.77	.85	1.1	1.5	90	140	140	110	105	108
A-22	0.3	1.4	1.6	1.0	1.25	72	172	118	172	150	142
A-28	0.4	1.1	1.2	.8	.96	260	168	170	200	195	176
A-38	0.3	1.6	2.3	1.0	1.1	130	220	132	175	175	150
A-39	0.3	1.8	2.15	3.0	3.6	12	30	16	42	50	50
A-41	0.5	1.8	2.05	1.1	1.2	22	32	15	55	84	92
A-42	0.1	1.0	1.23	1.15	1.27	8	98	90	68	68	70
A-45	0.15	2.2	2.75	1.1	1.26	36	200	190	92	190	180
A-48	0.35	-	-	0.85	0.9	-	-	-	115	126	150
A-49	0.4	-	-	0.35	0.35	-	-	-	200	108	105
A-50	0.2	-	-	0.55	0.63	-	-	-	77	145	122
A-53	0.3	-	-	1.08	1.38	-	-	-	130	135	122
Average	0.28	1.37	1.71	1.04	1.23	94	144	125	119	124	120
Series II											
B-24	0.2	0.7	0.88	0.9	1.16	30	172	168	205	195	200
B-26	0.65	2.33	2.82	1.12	1.42	205	192	180	210	210	205
B-49	0.35	0.90	1.15	0.60	0.65	128	165	148	170	160	150
B-52	1.00	2.00	2.40	2.00	2.34	150	160	160	208	195	192
Average	0.55	1.50	1.31	1.15	1.39	128	172	164	198	196	187

series II was less than in series I. Before vagotomy it was 2.7 times control and 2.1 times the control after vagotomy (Table I).

Only in one experiment, dog A-49, did a diuresis fail to follow an infusion of plasma. The depth of anaesthesia was deeper than usual in this animal and there was considerable blood loss.

Otherwise there was a consistent increase in urine flow of at least twice control level, following an infusion of heparinized plasma or 6% bovine albumin in Ringer-Locke solution (iso osmotic). Cutting the vagus nerves did not prevent the diuresis. The slightly smaller post-vagotomy diuresis could be attributed to possible deterioration in responsiveness of the animal.

Glomerular Filtration Rate

To correlate changes in renal function before and after an infusion of plasma or isosmotic bovine albumin, GFR's were plotted against controls on a scatter diagram (Fig. 2). Control values were plotted along the ordinate and along the abscissa was the GFR during the peak of the diuresis. A line at 45 degrees would represent a line-of-no-change. Each point represented on the graph was the average of two successive clearance periods.

In Fig. 2 it will be seen that most of the points are grouped around the line-of-no-change. Points below this

FIGURE 2

Scatter diagram showing changes in GFR during the diuresis in series I and II compared to control levels.

Ordinate:- control GFR levels.

Abscissa:- GFR during peak of diuresis.

Line at 45° is line-of-no-change and lines on either side of it indicate average deviation that occurs.

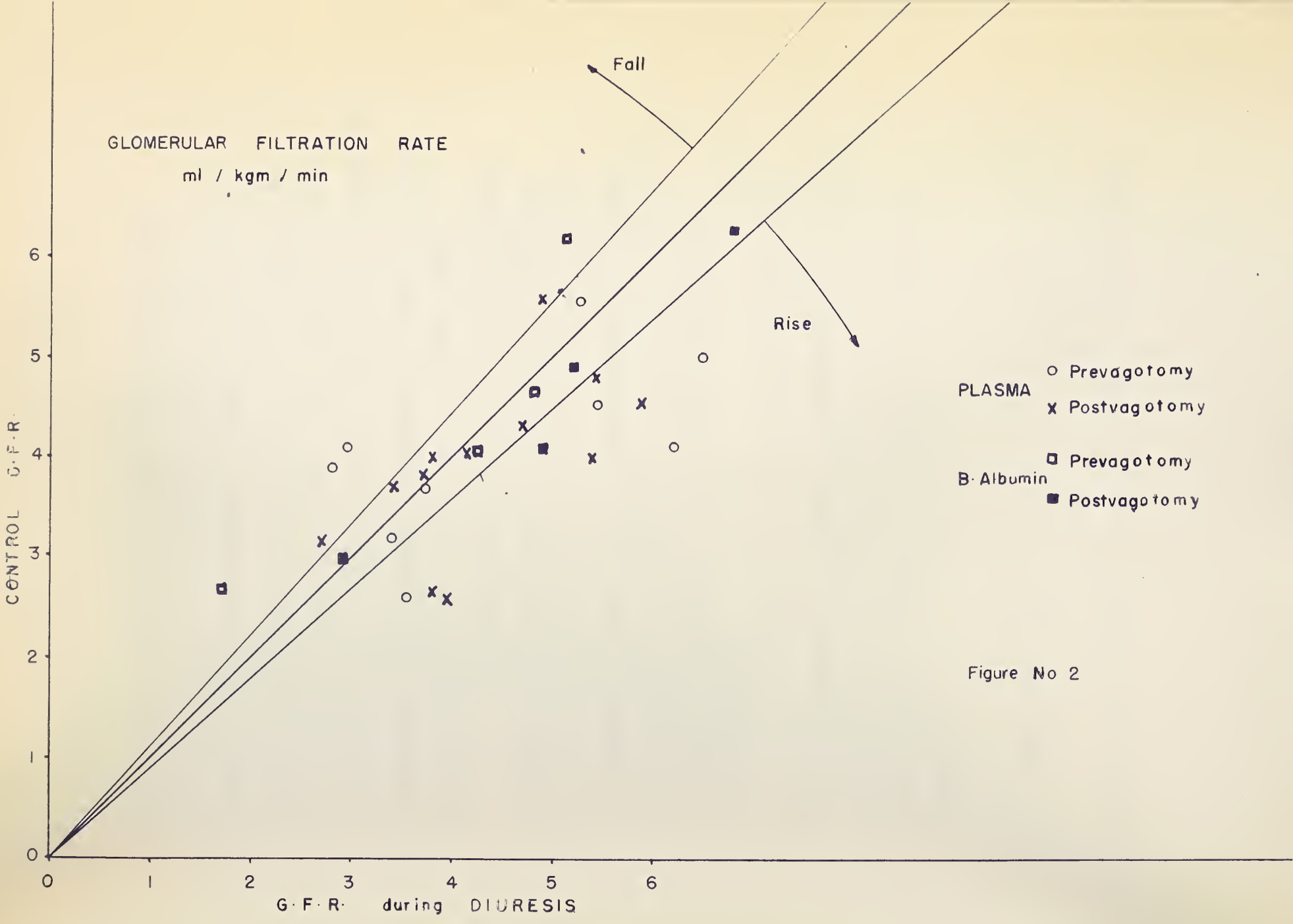

GLOMERULAR FILTRATION RATE
ml / kgm / min
Fall
Rise
PLASMA
Prevagotomy
Postvagotomy
B·Albumin
Prevagotomy
Postvagotomy
CONTROL G·F·R·
G·F·R· during DIURESIS
0
1
2
3
4
5
6

Figure No 2

line represent a rise in GFR over control and points above represent a fall. If the line-of-no-change is widened by allowing a 10% average deviation on either side, then even more points fall within the area-of-no-change. Of the points that fall outside this area, as many fall above as below and it must be concluded that no consistent change in GFR could be demonstrated. No difference can be seen between experiments using bovine albumin and those using plasma. Vagotomy had no apparent effect.

The average control GFR for 9 dogs before vagotomy in series I was 4.08 ml./kgm./min. and during the diuresis it was 4.44 ml./kgm./min. (Table II). This difference is probably not significant in the light of the 10% average deviation that occurs normally. After vagotomy the average control for 12 dogs was 3.94 ml./kgm./min. and during the diuresis it was 4.33 ml./kgm./min.

In series II the average pre-vagotomy control GFR was 4.4 ml./kgm./min. and during the diuresis was 4.0 ml./kgm./min. After vagotomy the average GFR during the diuresis was 4.9 ml./kgm./min. compared to a control of 4.6 ml./kgm./min. (Table II).

Renal Plasma Flow

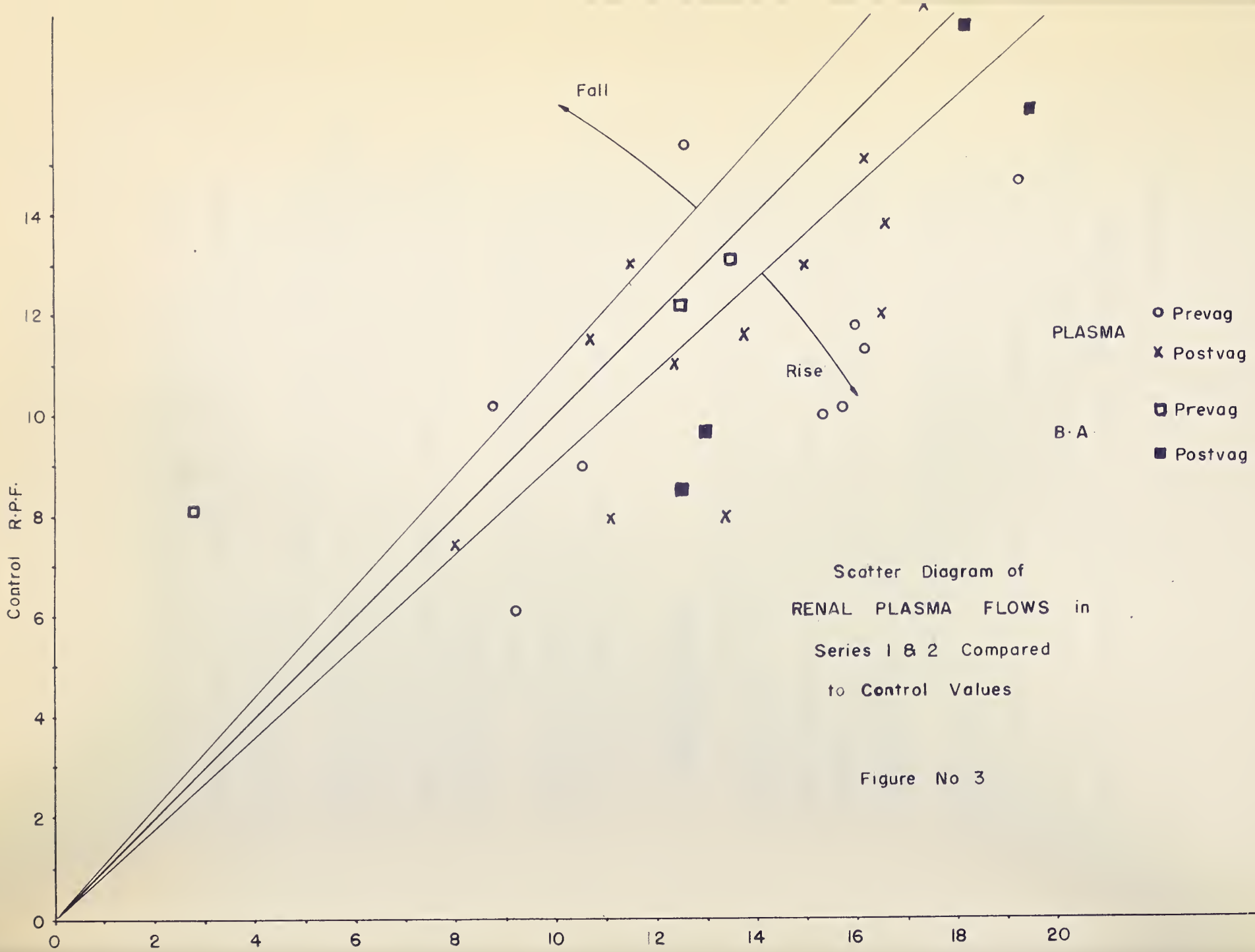
Values for the renal plasma flow (RPF) during the diuresis were compared with control values on a scatter diagram

Table II. Average renal function values during the diuresis compared to control levels.

	GLOMERULAR FILTRATION RATE				RENAL PLASMA FLOW			
	Pre-Vagotomy		Post-Vagotomy		Pre-Vagotomy		Post-Vagotomy	
Dog #	Control	During Diuresis	Control	During Diuresis	Control	During Diuresis	Control	During Diuresis
Series I								
A-18	5.56	5.30	-	-	10.2	8.76	-	-
A-19	3.21	3.39	3.73	3.43	6.1	9.2	7.37	8.0
A-21	4.53	5.48	4.8	5.46	11.3	16.2	13.0	11.5
A-22	4.1	6.2	4.55	5.9	11.8	15.95	13.0	15.0
A-28	5.0	6.55	5.57	4.9	14.74	19.29	18.7	16.8
A-38	3.69	3.75	3.8	3.73	10.0	15.35	11.6	13.8
A-39	-	-	4.03	3.8	-	-	11.5	10.7
A-41	4.1	2.95	3.15	2.7	15.4	12.6	11.0	12.35
A-42	2.64	3.54	4.0	5.36	9.0	10.5	11.95	16.55
A-45	3.9	2.80	-	-	10.14	15.76	-	-
A-48	-	-	4.08	4.29	-	-	15.1	16.2
A-49	-	-	4.31	4.69	-	-	13.8	16.6
A-50	-	-	2.64	3.80	-	-	7.9	11.1
A-53	-	-	2.63	3.98	-	-	7.96	13.4
Average	4.08	4.44	3.94	4.33	10.96	13.37	11.9	13,50
Series II								
B-24	6.2	5.12	6.3	6.8	22.2	14.7	17.8	18.2
B-26	4.64	4.8	4.1	4.9	12.2	12.5	9.65	13.0
B-44	2.7	1.70	3.0	2.95	8.1	2.8	8.5	12.5
B-52	4.08	4.24	4.92	5.21	13.2	13.55	16.2	19.5
Average	4.40	3.96	4.58	4.96	13.90	10.88	13.03	15.80

FIGURE 3

Scatter diagram showing changes in RPF during the diuresis in Series I and II compared to control levels. Line at 45° is line-of-no-change and lines on either side of it indicate average deviation that occurred.



Scatter Diagram of
RENAL PLASMA FLOWS in
Series 1 & 2 Compared
to Control Values

Figure No 3

in a similar manner to that for the GFR. (Fig. 3). An 11% average deviation from the mean was also plotted which widened the area-of-no-change.

It will be noted that there is a tendency for the points in both series I and II to fall below the line-of-no-change. This indicates a rise in the RPF during the diuresis. It will also be noted that the scatter is much greater because individual variations are greater.

Table II shows that the average RPF during the diuresis is consistently higher than control values in series I and II and cannot be accounted for by normal variation.

Sodium Excretion

The diuresis following an infusion of plasma or isosmotic bovine albumin was always accompanied by an increased sodium excretion. It will be noticed in plots of typical experiments (Figs. 9-14) that the total sodium output closely parallels the urine output.

Sodium concentrations were plotted on a scatter diagram (Fig. 4) and arrows were used to indicate the direction of the change in concentration from the beginning to the peak of the diuresis compared to an arbitrary control value which was the concentration immediately before the start of the infusion. An arrow pointing to the left indicates a fall in concentration

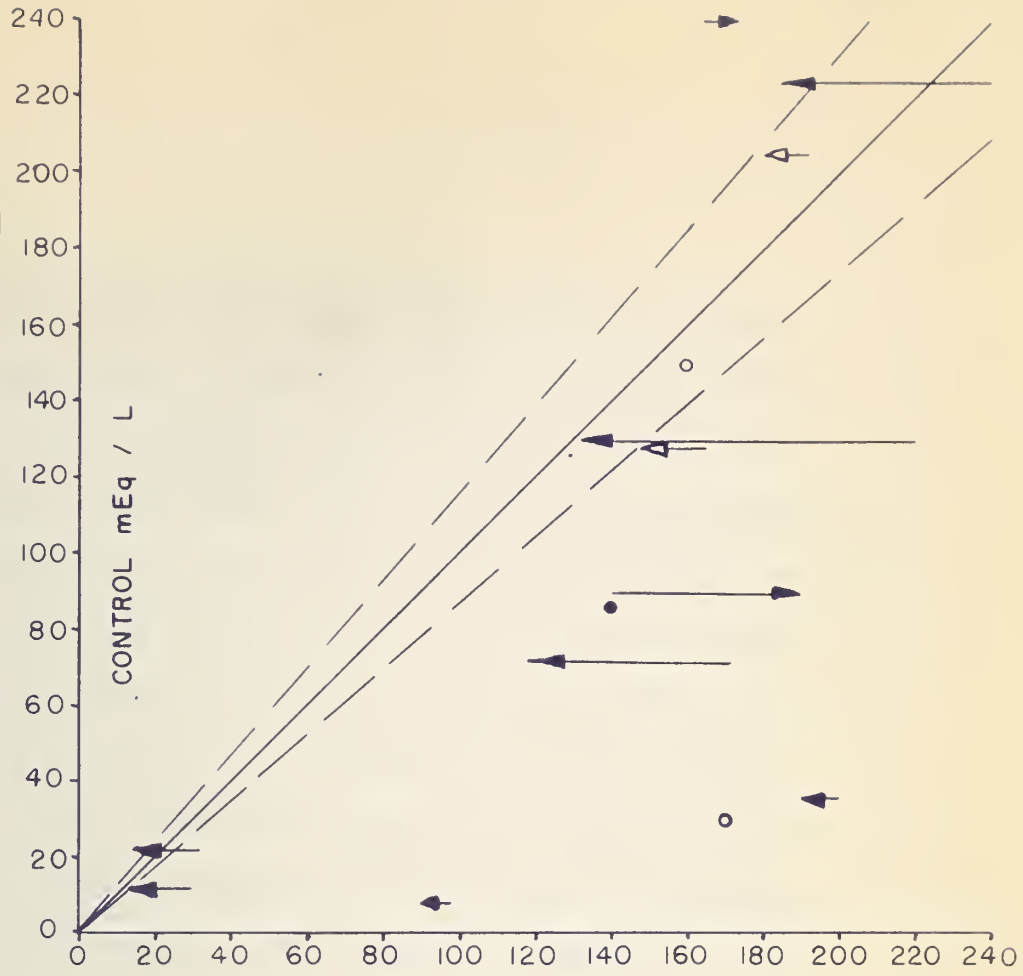
FIGURE 4

Scatter diagram comparing changes in urine sodium concentration before vagotomy at the start and the peak of the diuresis to control levels. Arrows indicate the direction of the change that took place from the beginning to the peak of the diuresis. Line at 45° is line-of-no-change. Dotted lines represent average deviation.

FIGURE 5

Scatter diagram comparing changes in urine sodium concentration after vagotomy at the start and the peak of the diuresis to control levels. Arrows indicate the direction of the change that took place from the beginning of the diuresis to the peak. Line at 45° is line-of-no-change. Dotted lines represent average deviation.

URINE SODIUM CONCENTRATION BEFORE VAGOTOMY



● PLASMA ◀

○ BOVINE ALBUMIN ◁

FIGURE 4

URINE SODIUM CONCENTRATION AFTER VAGOTOMY

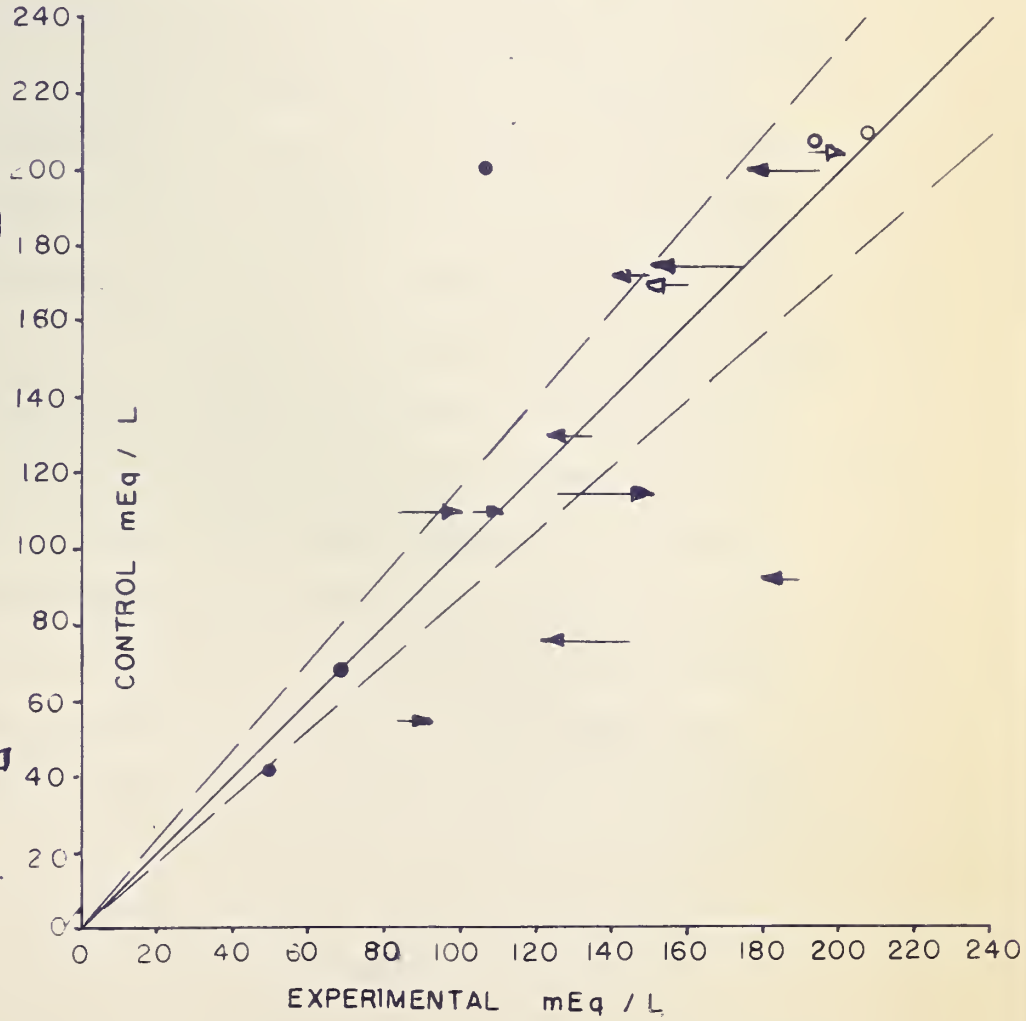


FIGURE No 5

● PLASMA ◀

○ BOVINE ALBUMIN ◁

and the length of the arrow represents the amount of change. A single point without an arrow shows that no change occurred.

In those animals with intact vagi in series I and II, the sodium concentration rose with the start of the infusion and then fell when the peak of the diuresis was reached. Therefore in the scatter diagram (Fig. 4) most of the points are below the line-of-no-change and the arrows point to the left.

After vagotomy the infusion had little effect on the urinary sodium concentration and as can be seen in Fig. 5 most of the points are grouped around the line-of-no-change. This meant that there was little change in concentration from the start of the diuresis to the peak and hence all the arrows are short. Vagotomy was usually done after a preliminary infusion and in order to determine any effect that this might have had, four dogs were vagotomized early in the experiment before any procedure was done. The results from these four dogs did not alter the previous findings, as the sodium concentration also failed to rise significantly in these animals.

To estimate the variation that normally occurs between two successive urine sodium concentration readings, random pairs of readings were taken during the control period of the animals in series I. The average deviation from the mean of these readings was expressed as a percentage and plotted on either side of the line-of-no-change in the scatter

diagrams. The area between roughly represented the normal variation that might occur before changes became significant. Thus, in Fig. 5 most of the changes in concentration can be explained as normal variation.

In a few experiments in which a diuresis did not result from an infusion or appeared late (see Fig. 12), it was noticed that there was a slow rise in sodium concentration without a change in urine volume.

It was also an impression in a few of the experiments that the sodium concentration slowly rose following vagotomy. This was not a consistent finding and appeared in only one control animal.

Effect of Antihistamines

Four animals were given 0.5 mgm./kgm. of a mild antihistamine, Chlor-Tripolon (Schering) in the infusate to rule out the possibility that histamine (recently shown to be liberated from separated plasma in the dog (McIntosh, personal communication)) might be causing the diuresis. The increased urine flow followed the infusion as usual and no changes were noted.

Effect of Hemorrhage

In nine experimental animals, 50 to 100 ml. of blood was removed in an effort to reduce the diuresis to control levels. If the blood was removed over a period of 10 to 15 minutes, the blood pressure was not affected but the urine flow fell rapidly (see plot of experimental data, Fig. 10). The GFR and RPF were temporarily reduced in every case but on no occasion was there any decrease in sodium concentration. In two cases, the concentration rose slightly. There would seem to be two effects; a rapid arteriolar constriction and a slower change in tubular reabsorption.

Arterial and Venous Pressure

Relationship between Arterial Blood Pressure and Urine Flow

No consistant relationship could be found between increases in arterial blood pressure and changes in urine flow. The infusions were given slowly enough to have little effect on the blood pressure and yet large diureses resulted. One consistent finding however, was that once the blood pressure fell below 60 mm. of Hg, urine output ceased.

There were many instances in which urine flow increased without any change in blood pressure and conversely

the blood pressure often rose or fell without altering the urine flow (see Fig. 9). There was usually a sharp temporary increase in blood pressure following vagotomy but this seldom affected the urine flow (Figs. 11 and 14).

Relationship between Right Atrial Pressure and Urine Flow

There was a rough correlation between increases in right atrial pressure and urine flow. The infusion usually caused the venous pressure to rise and this was generally followed by a diuresis but there were several instances in which the venous pressure did not rise and it was these exceptions which threw doubt on the hypothesis that the increased urine was the result of the increased right atrial pressure.

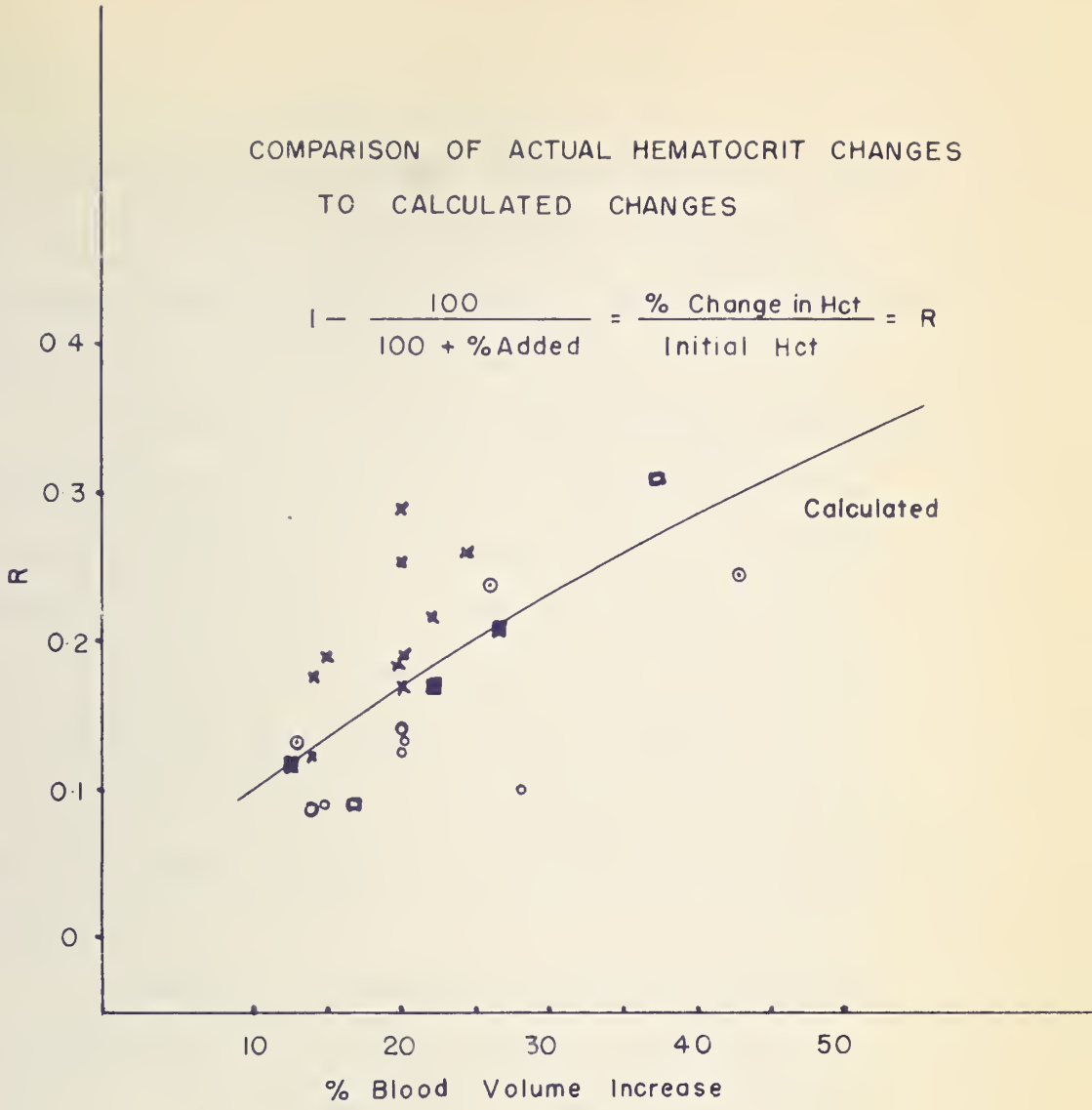
In the representative protocols, dog A-38 (Fig. 9) shows a large increase in urine flow with very little change in right atrial pressure. Other isolated examples of the same phenomenon can be found in the series. The peak of the rise in the venous pressure when it occurred, preceded the peak of the diuretic response by about 20 minutes.

Hematocrit Changes.

Figure 8 shows a graph with a line along which are the changes in hematocrit that would theoretically occur for

FIGURE 8

A graph comparing actual hematocrit changes following plasma infusion with calculated changes for various per cent increases in blood volume. The scale along the ordinate was calculated from the stated formula in order to give a line that was nearly straight. Points around the line are actual hematocrits that resulted from plasma infusion.



PLASMA — Prevagotomy x
Postvagotomy o

BOVINE ALBUMIN — Prevagotomy ⊙
Postvagotomy □

POSTVAGOTOMY without preliminary infusion ⊙

given increases in plasma volume. Along the ordinate, percent changes in hematocrit over initial hematocrit were plotted. This allowed a smooth curve to be drawn. The points around this curve are the actual changes that took place.

Following the first pre-vagotomy infusion, the actual hematocrit values fell below this line which indicated that the change or dilution of the red blood cells was less than expected. It was noticed that by the end of an hour, the hematocrit had returned to its original level. The second or post-vagotomy infusion of plasma produced decreases in the hematocrit which were greater than expected. The red cells became more diluted than could be accounted for by the second infusion.

To study the possibility that these changes were due to conditioning by the first infusion, vagotomy was done early in the experiment without a preliminary infusion. This produced changes in the hematocrit which were close to those calculated.

When 6% bovine albumin in Ringer-Locke solution was used as the volume expander, the hematocrit changes were always less than those produced by a comparable plasma infusion.

Adrenalectomized Animals

This series consisted of seven adrenalectomized dogs which were maintained on cortisone and desoxycorticosterone acetate (DCA). The animals tended to deteriorate rapidly under anaesthesia and often the experiments could not be completed. It could be shown however, that a diuresis always followed a plasma infusion before vagotomy. Two failed to show any increase in urine flow after vagotomy but one of these had deteriorated markedly.

The average control urine flow was 0.25 ml./min. (see Table III) and the first infusion was followed by a four-fold average increase in urine output in six animals. The second infusion, after vagotomy, caused only an average urine increase of 2.24 times control values. This was undoubtedly due, in part at least, to the animal's poor condition by the end of the experiment. The plasma infusion frequently caused a vascular collapse and renal shutdown which resembled anaphylactic shock. On two occasions continued transfusion restored the dog's blood pressure to near normal values and urine production resumed. In one case in which an antihistamine, Chlor-Tripolon was used, the collapse did not occur.

Renal function tests were available for only 5 experimental animals (see Table III). In three dogs, GFR

Table III. Adrenalectomized dogs.

Dog	URINE FLOW					URINE SODIUM CONCENTRATION					
		Pre-Vagotomy		Post-Vagotomy			Pre-Vagotomy			Post-Vagotomy	
	Average control	Average diuresis	Peak	Average diuresis	Peak	Control immed. before infusion	At start of diuresis	At peak of diuresis	Control immed. before infusion	At start of diuresis	At peak of diuresis
D-25	0.25	1.22	1.78	0.2	0.2	195	220	180	95	52	40
D-27	0.25	0.65	0.80	0.40	0.42	200	176	135	38	50	70
D-29	0.22	0.80	0.85	0.40	0.44	224	205	64	10	12	16
D-30	0.15	0.60	0.70	0.59	0.73	200	84	84	20	50	32
D-31	0.25	1.50	2.07	0.72	0.79	40	10	30	50	44	50
D-34	0.20	0.80	0.98	1.00	1.30	8	8	8	30	28	12
D-51	0.40	1.62	2.08	0.50	0.60	115	64	40	3	3	3
Average	0.25	1.06	-	0.56	-	139	109	77	35	34	34

Dog	GFR ml./min./kgm.				RPF ml./min./kgm.			
	Pre-Vagotomy		Post-Vagotomy		Pre-Vagotomy		Post-Vagotomy	
	Control	During diuresis	Control	During diuresis	Control	During diuresis	Control	During diuresis
D-25	5.82	5.37	3.85	6.1	13.4	15.9	10.9	17.3
D-27	5.3	5.35	4.9	5.1	12.2	18.55	16.8	20.8
D-29	2.41	4.60	-	-	2.31	11.58	-	-
D-30	1.95	2.45	-	-	2.95	5.25	-	-
D-51	2.31	2.97	1.83	1.80	3.28	7.75	4.40	12.37
Average	3.55	4.15	3.53	4.33	6.83	11.80	10.70	16.82

FIGURE 6

Scatter diagram comparing changes in glomerular filtration rate and renal plasma flow in adrenalectomized animals with control levels. Line at 45° is line-of-no-change.

FIGURE 7

Scatter diagram comparing changes in urine sodium concentration in adrenalectomized dogs at the start and peak of the diuresis to control levels. Arrows indicate the direction of the change that took place from the beginning of the diuresis to the peak. Line at 45° is line-of-no-change.

SCATTER DIAGRAM OF RENAL FUNCTION TESTS AND URINE SODIUM CONCENTRATION OF ADRENALECTOMIZED DOGS COMPARED TO CONTROL VALUES

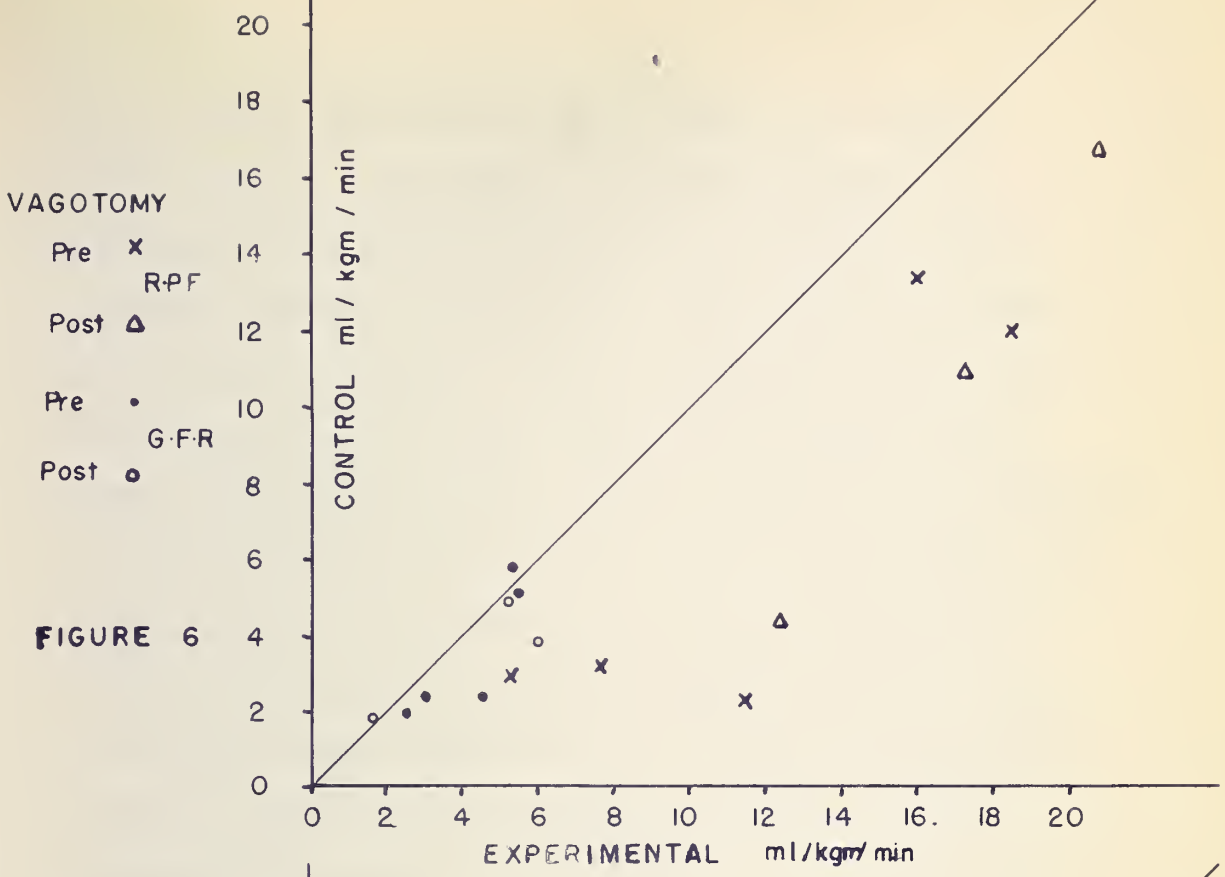


FIGURE 6

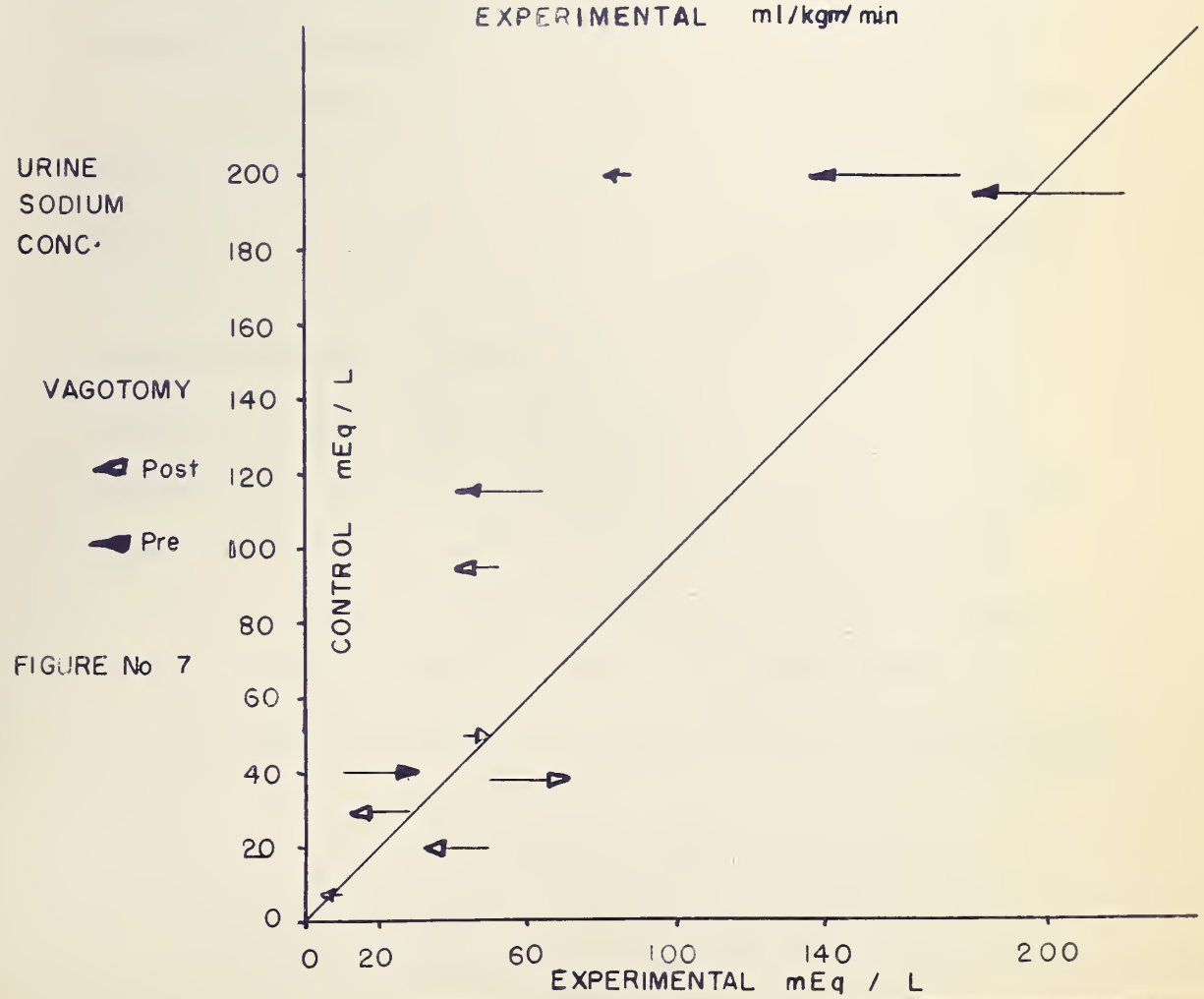


FIGURE No 7

control values were about 50% below normal. The average control GFR for 5 experiments was 3.55 ml./kgm./min. and during the first diuresis rose to 4.15 ml./kgm./min. After vagotomy, there was a similar rise in GFR, from 3.53 ml./kgm./min. to 4.33 ml./kgm./min. during the diuresis. These values were averages and individual values often rose much higher. Control RPF's were also much lower than normal (6.83 ml./kgm./min.) but during the diuresis they rose to nearly normal levels. This marked increase in GFR and RPF was not seen in normal dogs. GFR and RPF during the diuresis are compared to control levels in the same scatter diagram (Fig. 6). Most of the points fall below the line-of-no-change, showing the increase in renal function that occurred.

Changes in sodium concentration were plotted against control levels on a scatter diagram (Fig. 7) such as used in series I and II. Almost all the points fell above the line-of-no-change which indicated a fall in concentration. This meant that the increases in water output were greater than the increase in sodium output. Most of the arrows pointed to a decreased concentration at the peak of the diuresis. The total urinary sodium output showed some increase but not as large as with non-adrenalectomized animals, so not all the sodium response was lost. Table III shows that control levels of sodium concentration compared favorably with non-adrenalectomized animals.

FIGURE 9

Plot of experimental data from animal A-38 using plasma as the blood volume expander (20% of blood volume). 50 ml. of Ringer-Locke solution were used to prime the animal. The effect of two infusions is shown, the second one, after vagotomy. The effect of 25 ml. hemorrhage is also shown. Note the fall in arterial blood pressure with the first diuresis.

FIGURE 10

Plot of experimental data from animal A-28 in which 6% bovine albumin in Ringer-Locke solution was infused. The blood volume was expanded by a total of 30% and no diuresis resulted. The increase in urinary sodium concentration that followed is well demonstrated.

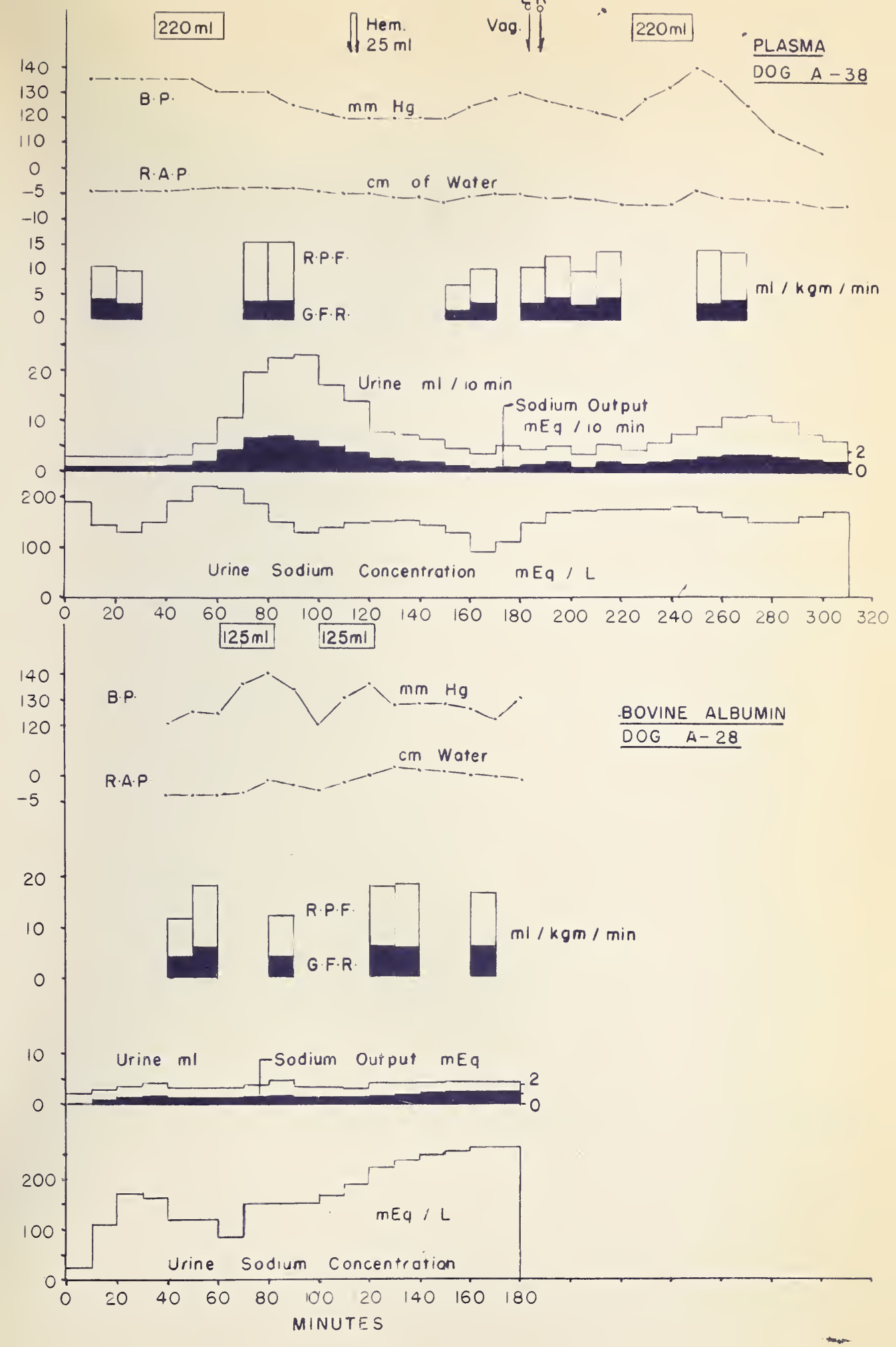
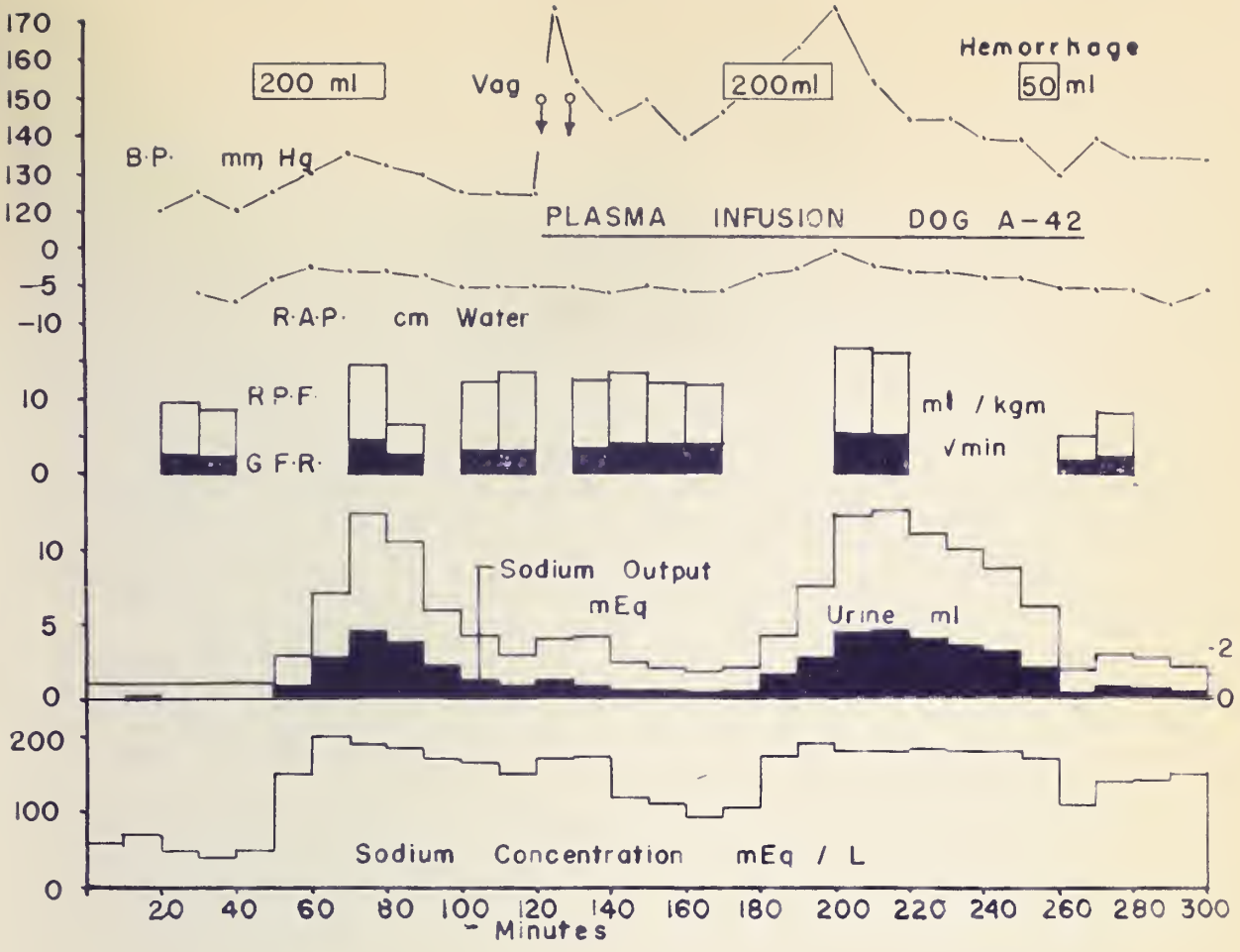

220 ml
Hem.
25 ml
Vag.
220 ml
PLASMA
DOG A-38
B.P.
mm Hg
R.A.P.
cm of Water
R.P.F.
G.F.R.
ml / kgm / min
Urine ml / 10 min
Sodium Output
mEq / 10 min
Urine Sodium Concentration mEq / L
125 ml
125 ml
BOVINE ALBUMIN
DOG A-28
Urine ml
Sodium Output mEq
mEq / L
MINUTES

FIGURE 11

Plot of experimental data from animal A-42. Two infusions of plasma (20% of blood volume) produced diuresis before and after vagotomy. Note rise in urinary sodium concentration with the start of the diuresis.

FIGURE 12

Plot of experimental data from control animal C-4. A slow infusion of Ringer-Locke solution raised the sodium concentration to a higher constant level.


200 ml
Vag
200ml
Hemorrhage
50 ml
B·P· mm Hg
PLASMA INFUSION DOG A-42
R·A·P· cm Water
R·P·F·
G·F·R·
ml / kgm / min
Sodium Output mEq
Urine ml
Sodium Concentration mEq / L
Minutes

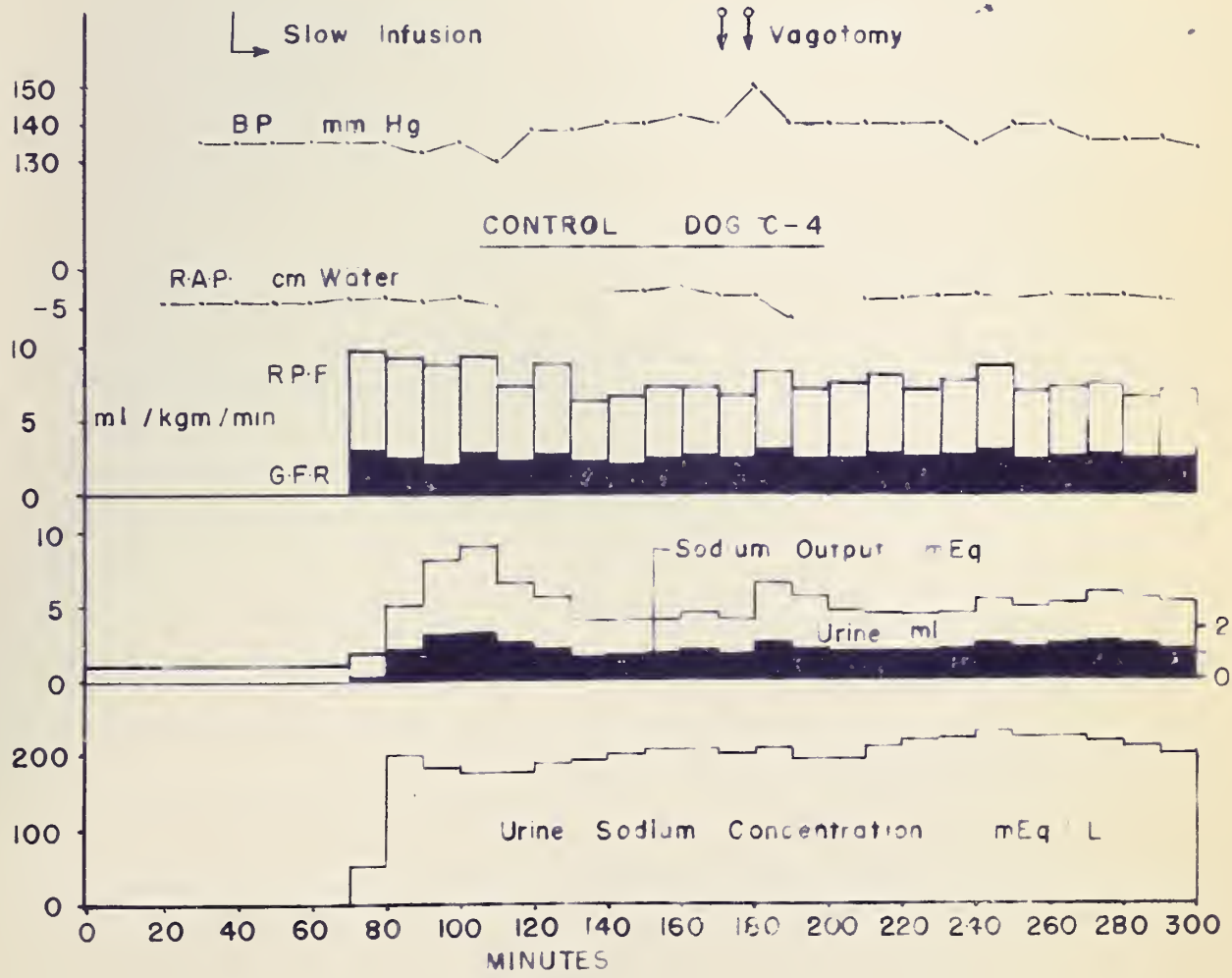

Slow Infusion
Vagotomy
BP mm Hg
CONTROL DOG C-4
R·A·P· cm Water
R·P·F
ml / kgm / min
G·F·R
Sodium Output mEq
Urine ml
Urine Sodium Concentration mEq / L
MINUTES

FIGURE 13

Plot of experimental data from adrenalectomized dog D-27. Note the fall in urine sodium concentration with the onset of the diuresis.

FIGURE 14

Plot of experimental data from animal B-26, using 6% bovine albumin in Ringer-Locke solution as the infusate.

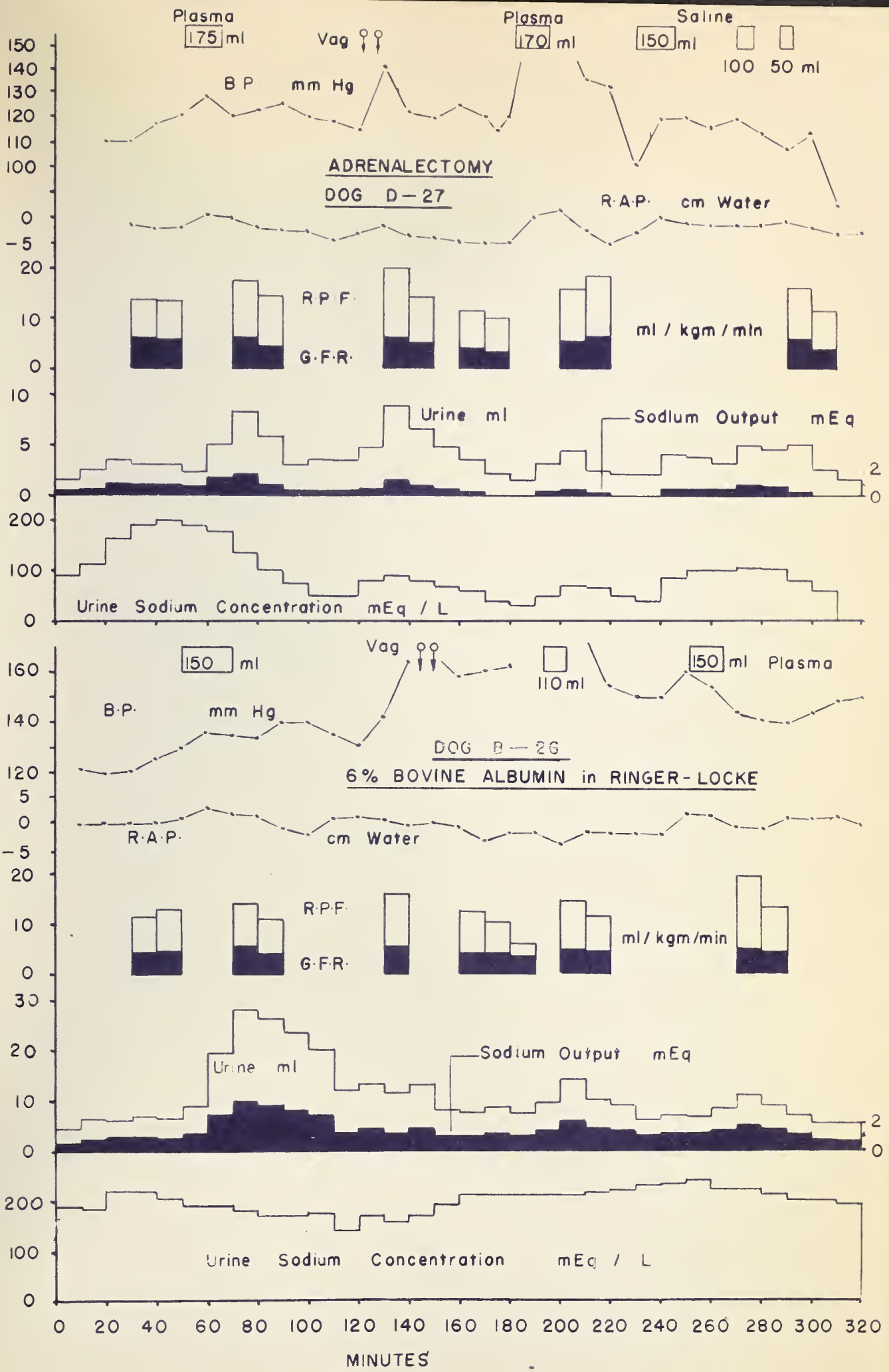
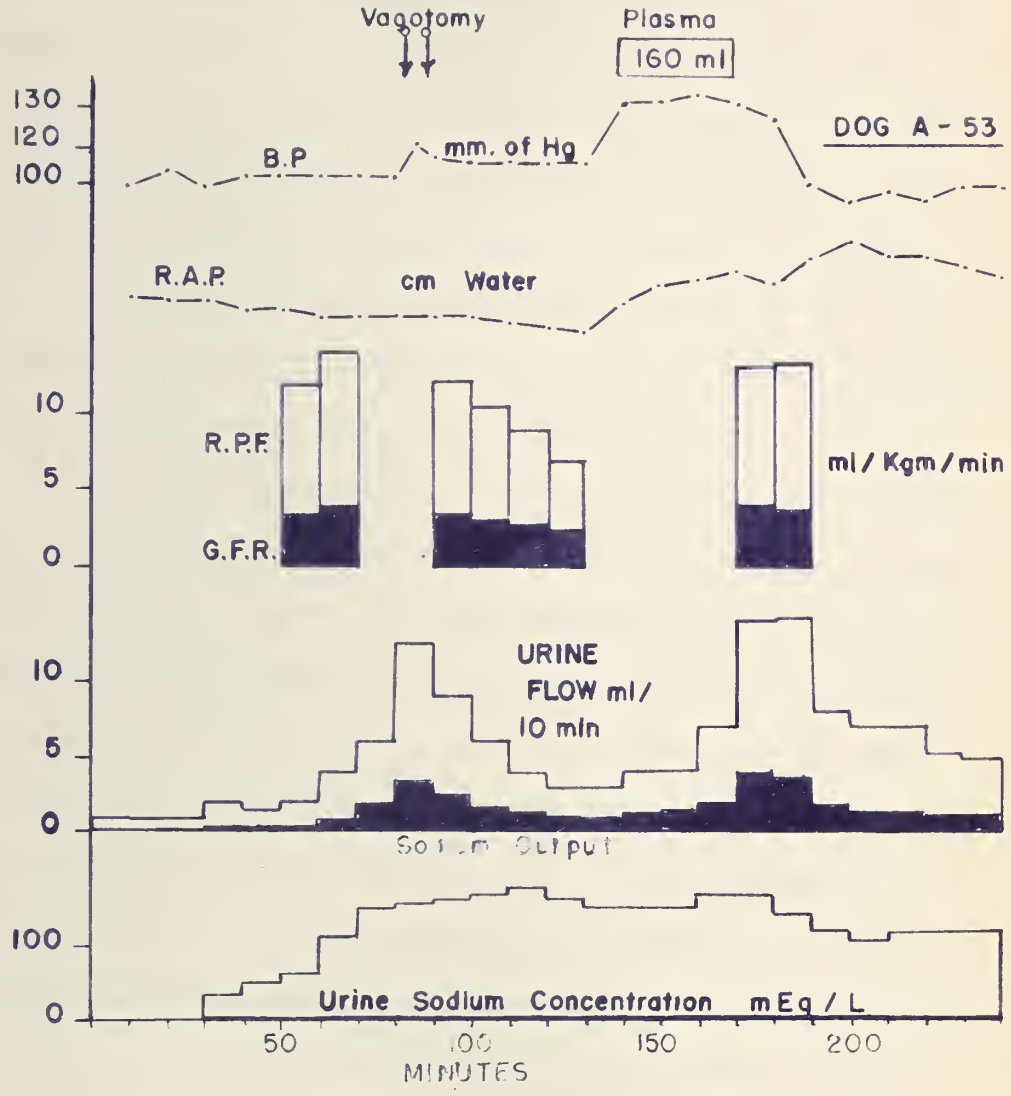

Plasma
175 ml
Vag
Plasma
170 ml
Saline
150 ml
100 50 ml
B P mm Hg
ADRENALECTOMY
DOG D—27
R·A·P· cm Water
R·P·F·
G·F·R·
ml / kgm / min
Urine ml
Sodium Output mEq
Urine Sodium Concentration mEq / L
150 ml
Vag
110 ml
150 ml Plasma
B·P· mm Hg
DOG B—26
6% BOVINE ALBUMIN in RINGER-LOCKE
R·A·P· cm Water
R·P·F·
G·F·R·
ml / kgm / min
Urine ml
Sodium Output mEq
Urine Sodium Concentration mEq / L
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320
MINUTES

FIGURE 15

Plot of experimental data from animal A-53 in which vagotomy was done before any other procedure and followed by an infusion of plasma containing an antihistamine, Chlor-Tripolon.


Vagotomy
Plasma
160 ml
DOG A - 53
130
120
100
B.P
mm. of Hg
R.A.P.
cm Water
10
5
0
R.P.F.
G.F.R.
ml / Kgm / min
10
5
0
URINE
FLOW ml/
10 min
100
0
Urine Sodium Concentration mEq / L
50
100
150
200
MINUTES

DISCUSSION

Before any conclusions were drawn from the renal function measurements an attempt was made to estimate the instrumental error and the average range of normal variation. The instruments themselves were shown to be reliable and the methods for determining creatinine and PAH were acceptable as can be seen from the recovery curves of Fig. 1. By using calibration curves calculated from plasma and urine, consistent errors in the method were almost eliminated.

To state whether or not a change in renal function was statistically significant was difficult. The lack of more than one pair of control readings made such analysis of the data within individual experiments unprofitable. It was also impractical to compare statistically one animal with another because of the significant difference between control values. The expected range of variation within one experiment, as determined from the control series, did however allow some judgment of the occurrence of change.

As was mentioned earlier, the average control values from renal function tests compared closely with those quoted by Smith (1951) for the dog. The urine sodium output was quite variable and no conclusions were based on absolute levels. One feature of the control experiments does require

comment and this was the occurrence of a definite but temporary diuretic response following vagotomy in more than half the control experiments. This result is opposite to that which would be expected from application of the hypothesis outlined earlier. If the vagi contained efferent fibers controlling tubular reabsorption of water, for which there is little or no evidence, it would be expected that there would be no diuretic response to infusion after vagotomy, which there is. It is probable, in view of the temporary nature of the response, that vascular factors or possibly the release of adrenaline, might be involved, despite the fact that no consistent changes in GFR or RPF were recorded in this series.

Consideration of the results can fall under three headings. First an attempt will be made to explain the findings in the light of previous knowledge and the hypothesis. A section follows in which speculation on changes in the hypothesis brought about by the results as well as new ideas on effector mechanisms are presented. Finally there will be a brief discussion of the direction in which future work might take. Under the first heading, the value of plasma as a volume expander, afferent pathways and effector mechanisms of a reflex controlling blood volume will be discussed.

Plasma as a Blood Volume Expander

Despite the fact that the plasma was withdrawn from the donor dog until complete exsanguination and must have contained high concentrations of anti-diuretic hormone (ADH), it consistently gave a diuresis. The amount needed to produce a diuresis varies from 10 to 43% of the total blood volume and seemed to depend on the animal's general health and state of hydration.

No significant difference could be found between the effects of plasma as a blood volume expander and 6% bovine albumin in Ringer-Locke solution except that the latter produced increases in urine flow that were not as great as with plasma.

Initial decreases in the hematocrit were not as great with the first infusion of plasma as would be expected (see Fig. 8). This implied that either the plasma rapidly leaked into the extracellular spaces and was trapped in small vessels in vascular reservoirs or else splenic reserves of cells were drawn upon. By the end of an hour, the hematocrit had nearly returned to control levels. The post-vagotomy infusion produced decreases in the hematocrit that were greater than expected. When vagotomy was followed by an infusion without a preliminary infusion, the results were as expected. This would seem to indicate that the vagi carry impulses which

affect either the distribution of the blood or the permeability of the vessels to regulate the hematocrit. However, no conclusion can be drawn to explain this very interesting phenomenon.

Bovine albumin infusion made by adding 6% of relatively sodium-free bovine albumin powder to Ringer-Locke solution, was shown to be less efficient than plasma as a blood volume expander. Hematocrit changes were lower than expected in most cases compared to those following plasma, even after vagotomy. This could account for the smaller urine volumes that resulted.

Bovine albumin was presumably free of physiological substances such as anti-diuretic hormone, and yet it produced a diuresis which was very similar to that following infusions of plasma.

Contribution of Vagal Pathways to the Diuretic Response

There is good evidence that expansion of the blood volume causes a diuresis although the pathways of this reflex are unknown. It has been suggested (Henry and Gauer) that atrial stretch receptors are blood volume receptors and that the vagus forms the afferent pathway of such a reflex, resulting in changes in urine flow. Information from this investigation has added evidence to the work showing that physiological increases in blood volume by plasma infusion cause a diuresis.

A plasma infusion would likely distend the atria and has been shown almost invariably to cause a diuresis. The quality of the diuresis is not quite the same as when mechanical stimulation of the atria is used, for this causes primarily a water diuresis while an iso-osmotic infusion produces a saline diuresis. This would suggest that the two mechanisms are not the same.

There was a rough correlation between increases in right atrial pressure (RAP) and urine volume and it was thought by some workers (Henry, et al.) that this demonstrated that infusion stimulates the atrial stretch receptors and thus causes the diuresis. However, there were several experiments in which the RAP did not rise during the diuresis or rose very little and these exceptions place doubt on the theory. Nevertheless, it has been suggested (Paintal, 1953 and Pearce, et al., 1956) that pressure is not the stimulus for the atrial receptors but that distention is. Pressure in the atrium may increase while the tone of the walls prevents stretching. Also, they showed electrophysiologically that infusion increases afferent discharge in atrial receptors.

Further evidence was accumulated which helps to render untenable the theory that systemic blood pressure influences urine flow. Although urine flow increases sometimes following blood pressure rises, there were many instances in which this did not happen or the reverse occurred.

Impulses from atrial stretch receptors are carried to the brain via the vagus nerve and if the hypothesis is true, then cutting these pathways would interfere with blood volume regulation. It has been shown that vagotomy does not interfere with the increased water response of a blood volume expansion. Reports (Pearce and Henry, unpublished) indicate that other pathways from the atria are extremely unlikely. Thus, only two conclusions seem possible; either atrial receptors play no part in the physiological control of blood volume or there are other volume receptors the afferent pathways of which are not in the vagus nerve.

An analysis of the events following a plasma infusion gave information on the mechanisms influencing urine flow. The first response to an increased blood volume was a marked increase in the concentration of sodium in the urine. Twenty minutes after the start of the infusion, the sodium concentration then fell and was accompanied by a large increase in water output. This would seem to indicate that two separate mechanisms are operating. The earlier and faster response is an outpouring of sodium which is followed by an increased water output a few minutes later. There is additional evidence that the excretion of sodium and water are controlled by separate mechanisms. In a few cases (see Fig. 10, dog A-28) the diuresis

failed to occur until the blood volume had been expanded by a much larger amount than was usually necessary. Presumably these animals were malnourished and dehydrated and had a low blood volume. It can be shown in these cases that the sodium concentration in the urine gradually increased while the urine volume remained the same. This was also evidence against the concept that the increased urine flow was only an osmotic diuresis, that is, water being excreted as a result of the decreased tubular reabsorption of sodium.

It is doubtful if a diuretic substance in the infused plasma was the cause of the increased urine flow because a diuresis still followed iso-osmotic bovine albumin infusion. Bovine albumin presumably would not contain any such substance. Histamine was also eliminated as a cause for the diuresis when an antihistamine failed to prevent a diuretic response to infusion.

There is some evidence to suggest that although vagotomy did not affect the excretion of water, it did change the sodium response. When plasma was infused after vagotomy, the marked increase in urine sodium concentration followed by decrease, failed to occur. The concentration remained very constant throughout the diuresis. Because a previous infusion had been given before vagotomy, it could be argued that the first infusion had in some way conditioned the response. To

investigate this possibility, four dogs were vagotomized early in the experiment without having a preliminary infusion. A post-vagotomy plasma infusion produced little change in the sodium concentration (Fig. 15). This helped to support the view that the vagi carry impulses which, through some unknown mechanism, inhibit tubular reabsorption of sodium.

From these considerations it can be concluded that the atrial stretch receptors play only a minor role in the control of blood volume but there is a suggestion that they do have some effect on sodium excretion. In view of this, there must be other stretch organs responsible for control of water output in the regulation of blood volume, unless an entirely different type of mechanism is postulated. Most of the large veins possibly have this function as well as certain organs like the liver and spleen.

Effector Mechanisms of the Diuretic Response

The investigation of the effector mechanisms was studied through renal function tests and analysis of sodium excretion. Glomerular filtration rates were found to be essentially unchanged during the diuresis. The average variation with the same urine flow was 10% and there was considerable variation. The GFR even decreased during the diuresis in a few cases and this tended further to rule out change in filtration rate as being a factor in causing the diuresis.

There was a greater tendency for the RPF to increase during the diuresis and it was felt that although both the GFR and RPF were autonomously controlled, the RPF was probably the less stable. This slight increase in RPF would not have any great effect in itself on the production of urine. No evidence was found to support the view of Selkurt (1954) that small increases in GFR account for changes in the urine flow.

Sodium Response

In three of the adrenalectomized dogs, the control GF^R and RPF were low (about 50% of normal) and an infusion of plasma caused a marked increase in both to levels above normal. These were the only cases in which a detectable rise in the renal function values was discernible. It is possible that the adrenal cortex exerts some controlling influence over the GFR. The anterior pituitary at least, has been shown to influence glomerular function (White, et. al., 1949) possibly through its effect on the adrenal cortex. In the two animals that did not demonstrate this increase in renal function it was the opinion that the glands were not completely removed. An increased GFR does not entirely explain the diuresis in these cases however. A diuresis due to an increased GFR alone would be accompanied by a corresponding increase in sodium output. In these cases however, the sodium concentration decreased so the diuresis was not due solely to an

increased amount of filtrate presented to the tubules.

Although the seven experiments using adrenalectomized animals were not uniformly successful, they did yield valuable information about the effector mechanisms that control urine flow. These dogs were maintained on cortisone and DCA and were apparently healthy by the time of the experiment. Absence of the adrenal glands and a constant level of adrenal corticoids did not prevent a diuretic response to an increased blood volume. The natriuretic response was decreased however, because the sodium concentration fell during the diuresis. There was a slight increase in the total sodium excretion during the diuresis but it was considerably less than in normal animals. Rosenbaum, Papper and Ashley (1952) have also drawn attention to the fact that minor variations in the output of adrenal corticoids do not account for changes in the amount of sodium excreted. Patients with Addison's disease, maintained on a constant level of DCA and cortisone, could still vary their urinary sodium output, although the presence of cortisone at least was necessary for this phenomenon.

This is rather difficult to explain but it indicates that sodium excretion is not entirely dependent on the adrenal cortex although the presence of the corticoids is necessary. This would seem to suggest that all the mechanisms for controlling sodium excretion are not contained in the adrenals which would give added weight to the suggestion of Cort (1955) that sodium

excretion is under the influence of nerves supplying the tubules. No evidence was gained in this report to prove or disprove such a theory but the possibility of the existence of such a mechanism should be considered. How much of the natriuresis that results can be accounted for by sodium following the water diuresis, cannot be stated at this time but it would appear that this cannot explain all the increase in sodium excretion.

Adrenalectomy did have some effect on sodium excretion however. Loss of the natriuretic effect of blood volume expansion is difficult to explain when it is considered that all the known mineralo-corticoids are sodium-retaining in character. If blood volume expansion resulted in a decreased secretion of these hormones, the effect would be a slow rise in sodium excretion because the effects of aldosterone especially last for 1 to 2 hours. Such was not the case however, for the sodium response was immediate and appeared too soon to be accounted for by destruction of a sodium-retaining hormone. Therefore, this raises the possibility that the intact adrenal cortex might contain a sodium-losing hormone which is stimulated by an increased blood volume. That the natriuresis is largely controlled by the adrenals is shown by the fact that the sodium response is partly lost after adrenalectomy.

Considerable work has recently appeared in the literature suggesting that aldosterone is responsible for the

sodium retention of congestive heart failure (Davis, et al., 1956). The evidence is good that its secretion is increased in pathological conditions of increased blood volume and also increased in hemorrhage (Farrell, et al., 1956). There is insufficient evidence yet to state that it controls blood volume, although Barther, et al. (1956) have reported that its secretion is related to the extracellular fluid volume. Evidence from the investigation reported in this thesis has made decreased aldosterone an unlikely mechanism in the short-term control of blood volume. However, aldosterone still seems likely to affect extracellular fluid volume over long periods of time.

It is not proposed that a natriuretic hormone explains all the sodium response because regardless of the lack of adrenal cortex, sodium excretion is still increased to some extent. A clue to this might exist in the events following vagotomy. This procedure resulted in a diminished sodium response to infusion. The concentration remained stable although the total urinary sodium increased. One possible explanation for this is that the vagus represents the afferent limb of a reflex control of sodium excretion in response to increased blood volume. Against this are the experiments in which sodium excretion was not affected in response to mechanical stimulation of the atria (Pearce and Henry, 1954). These conflicting results cannot be reconciled at the present time but it may be that the vagi carry impulses from other receptor organs besides the atria and these influence sodium excretion.

A second possible explanation is that the vagus carries efferent fibers to the kidney as part of a reflex which affects tubular reabsorption of sodium. If this pathway is cut, presumably the natriuresis following an increased blood volume would not take place, as actually happens. However there is little evidence that the vagus supplies the kidney and even less that it affects tubular reabsorption.

The sodium retention of chronic congestive heart failure might be related to this suggested reduction in vagal impulses. Overdistention of the atria for long periods of time has been shown to result in a decrease in the number of impulses from the stretch receptors (Pearce, Henry and Chapman, 1956). In cardiac failure where the heart is dilated, the characteristic sodium retention could be explained by this decrease in impulses from the atria.

Water Response

Of the procedures done, viz., adrenalectomy and vagotomy, neither affected the water response to an increased blood volume. It seems safe to state that the adrenal gland has little to do with this. Furthermore, an isotonic and iso-osmotic infusion would not, as far as is known, affect the supraoptico-hypophyseal system which controls the osmotic pressure of the blood. It is possible, however, that ADH may be inhibited

as a result of an increased blood volume and there is some evidence that this is the case. Inhibition of ADH secretion would allow a fairly rapid disappearance of circulating hormone, perhaps equivalent to the time required for the onset of the diuresis. Inhibition of ADH as a result of an expanded blood volume has been suggested by several workers in the past (Gauer, et al., 1954). Most recently Cole (1957) came to the same conclusion following experiments with isotonic saline infusions in rats. This also offers a plausible explanation to the question of the water diuresis following plasma infusion. The infused plasma was undoubtedly high in ADH as it was obtained by exsanguination, yet this did not inhibit the urine volume increase. Variability in the degree of diuretic response might well depend on a competition between these effects.

There are two other explanations for the water diuresis that should be considered even if they are lacking in experimental evidence. Cort's suggestion (1954) that the nervous system directly controls tubular reabsorption of sodium, could conceivably apply to the reabsorption of water as well. There is almost no experimental evidence to support this however and sectioning of the renal nerves gives results which are difficult to interpret (see Marshall and Kolls, 1919 and Berne, 1952). Even more unlikely is the suggestion that a diuretic hormone

exists which is stimulated by an increased blood volume. It has been suggested that the growth hormone of the anterior pituitary antagonizes ADH but Homer Smith (1951) concluded in his review of the work on this that the weight of opinion does not support the existence of such a hormone. Certain unexplained phenomena such as the ability of diabetes insipidus dogs to excrete a water load (Shannon, 1942) need to be explained before such an hypothesis can be completely discarded.

In conclusion, if there are separate mechanisms for the excretion of water and sodium following an increase in blood volume, it seems likely that the posterior pituitary and the adrenal cortex at least play a part. A significant part of the sodium response is lost when the animals are adrenalectomized and it seems likely that this is due to the absence of some hormone. However, some of the natriuretic effect of an increased blood volume still remains despite the absence of all the adrenal corticoids except maintenance levels of DCA and cortisone. Some explanation for this must be sought outside the adrenals and the effect of vagotomy suggests that nervous influences possibly change tubular reabsorption of sodium. The mechanism for the water diuresis following plasma infusion also cannot be explained but there is the negative evidence that the vagus nerves and the adrenal glands are not involved. The occasional observation of decreased sodium concentration during the diuresis suggests that inhibition of ADH is a causative factor.

Effect of Hemorrhage on Renal Output

According to the hypothesis discussed earlier, hemorrhage should have an effect on the volume regulating mechanism that is opposite to that observed when the blood volume is expanded with plasma. That is, instead of stimulating hypothesized volume receptors, their action should be depressed resulting in a conservation of water and electrolytes. It is well known that hemorrhage increases the liberation of ADH but if other mechanisms are involved as well, sodium excretion may be affected. If the sodium control mechanisms suggested earlier to explain the increased total sodium output, operate in the reverse, the total sodium should decrease. It has been shown in this investigation that both water and sodium output decrease with bleeding although the urine sodium concentration does not change. This is explained by an equal reduction of water and sodium output. This could come about in two ways; a fall in filtration rate or a retention of sodium through some other mechanism as well as an increased reabsorption of water. That the GFR and RPF do fall was shown in this investigation but this is not the whole explanation, for the decrease in this renal function is only temporary and is outlasted by the fall in urine flow. It would appear then, that ADH does cause the anti-diuresis and a sodium mechanism, of which several were discussed earlier,

accounts for the fall in sodium output. Therefore, the hypothesis that separate mechanisms exist for water and sodium excretion is still possible and even more likely. It should also be noted that hemorrhage was done slowly enough so as not to affect arterial pressure significantly, so a fall in this parameter cannot be used to explain the antidiuresis.

It is not suggested that one factor controls blood volume but rather the combination of many sources of information interacting with several endocrine, nervous and mechanical effector mechanisms. Control of the constituents of the blood undoubtedly takes precedence over control of volume. Large changes in the blood volume can probably take place without serious effects on the animal. Perhaps the interstitial spaces are reservoirs for the excess extracellular fluid and changes in the permeability of arterioles allows more fluid to escape the vascular tree. The fullness of the vascular tree must be measured in some way and stretch receptors are the most plausible devices. These receptors are probably scattered throughout the vascular system and the information they send to the brain is analyzed somewhere in the hypothalamus. Changes take place in the breakdown of plasma proteins and red blood cells and in the excretion of water and electrolytes.

Finally, it should be emphasized that only the acute effects of altered blood volume have been discussed and that there

are undoubtedly long-term changes as well which take place over days or weeks. The reflex controlling blood volume is apparently a very complex one. To quote Claude Bernard, "True science teaches us to doubt and in ignorance, to refrain."

Suggestions for Future Experimental Approaches

The following thoughts have occurred to the author during the course of these investigations and are set down in the hope that future investigation in this line will be stimulated. Plasma as a volume expander was found to be good and had the only disadvantage that it left the vascular tree at a fairly rapid rate and lowered the hematocrit. Whole blood obviates these difficulties but has the added difficulties of hemolysis and incompatibility.

Although renal function tests in their present form are not accurate enough to measure small changes in GFR or RPF, their use, especially in an extended series of adrenalectomized animals where large changes in renal function were noted, seems warranted. Such a series would also provide added information on the effect of adrenalectomy. Before this could be done, a more satisfactory method for maintaining these animals should be sought; perhaps experiments without anaesthetic would help.

A series of experiments on hypophysectomized dogs would be very interesting especially since there is some suggestion that ADH may be inhibited by an increased blood volume. An additional test in future experiments might include a rat assay method for determining ADH content in the urine.

Finally, it would be very interesting to know the effect of vagotomy on the sodium retention of congestive heart failure.

CONCLUSIONS

(1) A diuresis followed an infusion of plasma or iso-osmotic bovine albumin solution in amounts of at least 15% of the calculated blood volume in 17 out of 18 dogs. The diuresis with bovine albumin was essentially similar but less than that with comparable amounts of plasma.

(2) The diuresis was accompanied by an increase in sodium output which was proportionally greater than the water output early in the diuresis (increase in urine sodium concentration) but less at the peak of the diuresis (decrease in urine sodium concentration). This response to the infusion, an initial increase in sodium output followed by an increased water output, was concluded to indicate altered tubular reabsorption of water and sodium. Other observations in the series supported the view that the diuresis and the natriuresis were controlled by separate mechanisms.

(3) The changes in GFR were variable and there was no apparent change with the diuresis but there was a tendency for the RPF to increase during the diuresis.

(4) Increases in right atrial pressure roughly corresponded to the diuresis but several exceptions to this were noted and it was felt that this relationship was not responsible for the diuresis.

Small increases in arterial pressure showed no consistent relationship to the diuresis.

(5) Vagotomy did not prevent the diuretic response to plasma or iso-osmotic bovine albumin solution but was usually associated with an altered sodium response.

(6) Because vagotomy did not alter the water response to plasma infusion, it was necessary to conclude that either atrial stretch receptors play no part in the physiological control of blood volume or there are other volume receptors, the afferent pathways of which are not in the vagus nerves.

(7) In a small series, a diuresis still occurred following plasma infusion in adrenalectomized animals maintained on cortisone and DCA, but the natriuretic response was decreased. Possible explanations for this partial loss of the sodium response to plasma infusion were discussed.

(8) Hemorrhage during a diuretic response caused a temporary decrease in GFR and RPF and increased the rate at which the urine flow approached control levels.

(9) Neither the vagus nerves nor the adrenal cortex is essential for the water diuresis that follows an increase in

blood volume. There were suggestions that inhibition of ADH is the mechanism by which this takes place.

(10) Possible effector mechanisms by which water and sodium excretion might be controlled in response to an altered blood volume were discussed.

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APPENDIX I

Cannulation of the Ureters of the Experimental Dogs.

This was done through an incision in the left flank, parallel to the tips of the transverse processes. The muscle layers of the external oblique, internal oblique and transversus were split along the direction of their fibers. The opening of the incision was then carried as far posteriorly as possible in order not to perforate the peritoneum and the remaining thin layer of fascia and fat was dissected. By spreading the incision, the peritoneum could be seen as a thin transparent membrane at the bottom of the incision. Usually the left ureter could be seen as a pink structure showing occasional peristaltic movements, just posterior to the opening. Before the left ureter was touched, two fingers were used to gently dissect the back wall through the loose retroperitoneal fibrous tissue to the right ureter. This was found much deeper and as soon as it was seen, two snaps were put on it as close to the bladder as possible. It was then divided between the snaps; the distal end ligatured and released. The left or upper ureter which was much easier to approach, was treated in the same way. Care was always taken to avoid pulling on the upper end of the ureter, as this was felt to be one cause of the henaturia which occasionally resulted.

Fine polythene cannulae (O.D. .038") were then inserted to approximately the pelvis of the kidney. They were then tied in

place firmly but care was used so as not to shut off the fine tubing. The grid-like incision was then allowed to close and the skin partly held together with a towel clip. The ureteral catheters were brought out at the lower end of the incision and allowed to drip into a 10 cc. graduated cylinder.

APPENDIX II

Operating Instructions for Flame Photometer

After the machine has been warmed up for an hour, the sensitivity control is set at the midpoint except when there is a lot of fluctuation or instability of the meter needle, in which case it should be turned one or two more revolutions in a clockwise direction. The selector switch is set at 0.1 and the meter needle is zeroed with the dark current dial. The wavelength dial is set at the sodium peak which was found to be 592 mu for this machine and then the blue phototube was positioned.

The tank oxygen pressure was set at 40 psi and the acetylene at 8 psi. The oxygen regulator on the control panel was opened to 10 psi, then the fuel regulator on the control panel is opened and the flame lit. The fuel pressure is then adjusted to provide a flame of about 2 to 2 1/2 inches in height, which is usually about 4 psi.

The most concentrated standard was then raised into position under the atomizer-burner. The shutter switch was turned to "ON" and with the transmittance dial set at 100% the meter needle was zeroed with the slit control knob. The other samples were then positioned and read as per cent transmittance. Frequent checks were made to zero the meter needle.

Before shutting off the instrument, a beaker of distilled water was aspirated before the flame was turned off. To shut off the instrument, the fuel tank valve regulator was turned off first, followed by the oxygen. A trickle charger was attached to the battery when it was not in use.

The burner often became plugged and failed to aspirate properly in which case special cleaning wires were inserted through the inlet of the capillary. If this did not remove the deposited material, a little diethyl ether was instilled into the oxygen inlet and blown through with oxygen. Careful handling of the burner was essential.

APPENDIX III

Care of the Adrenalectomized Dogs

The operative procedure of the adrenalectomization will not be described here except to mention the suitability of the transverse incision, but the pre- and post-operative care is as follows. Dogs were picked for their size (about 10 kgm.) and their apparent good health. On the day before the operation they were given 25 mgm. of intramuscular cortisone (Cortone, Merck & Co.) but no food or water was allowed on the day of the operation. During the operation, 500 ml. of normal saline containing 100 mgm. of hydrocortisone (Solu-Cortef, Upjohn) was slowly infused intravenously. Following the operation 25 mgm. of I.M. cortisone and 2.5 mgm. of desoxycorticosterone acetate (Percorten, Ciba) I.M. and the same on each subsequent day. One ml. of Dicrysticin was sometimes given I.M. if it was felt there was any chance of contamination of the wound. In addition, 4 grams of salt tablets were given daily per os. A special meat diet was also added. The animals recovered very quickly on this regime and by the second day were almost back to normal. Six days later the final experimental procedure was done.